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THE NUTRITIVE VALUE OF DIFFERENT SOURCES OF RICE BRAN FOR GROWING PIGS

BY

CHARLIE KAUFMANN

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A THESIS SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF **MASTER OF SCIENCE**

IN

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **THE NUTRITIVE VALUE OF DIFFERENT SOURCES OF RICE BRAN FOR GROWING PIGS** submitted by **CHARLIE KAUFMANN** in partial fulfillment of the requirements of the degree of **MASTER OF SCIENCE** in **ANIMAL SCIENCE.**

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ABSTRACT

An experiment was carried out to determine the apparent ileal amino acid digestibilities in five different samples of rice bran (**RB**) of various origins. In addition, the ileal digestibilities of amino acids associated with the neutral detergent fiber (**NDF**) fractions in the different sources of RB were also determined. Simple linear relationships were established between the apparent ileal digestibilities of amino acids and the NDF, crude fat (**CF**), ash, and crude protein (**CP**) contents in the RB samples, including and excluding RB 4 (an atypical source of RB). Simple linear relationships were established between the apparent ileal digestibilities of amino acids in RB and the content of CP associated with NDF in the RB-containing diets, including and excluding diet D, which contained RB 4.

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CHAPTER 1

GENERAL INTRODUCTION

Rice bran (**RB**) is a by-product of considerable quantity in countries where rice (*Oryza sativa*) is extensively cultivated. According to the USDA (2002), world production of milled rice amounted to 397,400,000 metric tons for the year 2000/2001. It is known that bran accounts for about 5% of the weight of brown rice (Juliano and Bechtel, 1985). A simple calculation reveals that there is the potential for the production of roughly 20 million metric tons of RB. Its widespread availability, coupled with the fact that cereal grains are too valuable to be used as animal feed in developing countries, makes RB a major constituent of animal diets in several countries. Rice bran is produced during the milling of brown to white rice, which occurs after the removal of the hulls. Approximately 20 % of the rice kernel by weight is attributed to the hull, and with the exception of silica, most minerals are present in insignificant amounts (Juliano and Bechtel, 1985). Hulls are low in protein, starch, and fat, but are high in fiber and ash (Pomeranz and Ory, 1982). Rice hulls have the lowest digestible content of nutrients of any cereal by-product, often less than 10 % (Juliano, 1985), and are therefore not an important component in diets for monogastric animals. In countries where grains are scarce, rice hulls are used mainly as a source of roughage in ruminant diets. The chemical composition of rice hulls is presented in Table 1-1.

Dehulling exposes the rice caryopsis of brown rice. Rice bran is composed of the outer four layers of the caryopsis, which are the pericarp, seed coat, nucellus, and aleurone, as well as much of the embryo. Five to eight % of the weight of brown rice is accounted for by the bran layers (Juliano and Bechtel, 1985), and it accounts for about 6

% of the rice paddy. The subaleurone layer and a small portion of the endosperm are removed during further milling of the caryopsis, and are collectively referred to as "polish." In the commercial production of white rice, the polish and bran layers are not separated, resulting in this entire milling fraction being called RB. Feed grade RB may also include various amounts of weed seeds, straw, hulls, and calcium carbonate, a milling aid. A powdered form of calcium carbonate is used in the polishing step to remove the germ and remnants of the bran, which is accomplished through abrasive action.

A. Chemical Composition and Digestibility of Rice Bran

Energy

Robles and Ewan (1982) determined the average energy values (kcal/g DM) for RB to be: gross energy (GE), 4.65; digestible energy (DE), 3.88; metabolizable energy (ME), 3.71, and net energy (NE), 2.03. The NRC (1998) reported values of 3.10, 2.85 and 2.04 kcal/g for DE, ME and NE, respectively. The GE values may vary between different sources, since these are dependent on the relative contents of carbohydrate, protein and fat. Using the aforementioned GE and DE values reported by Robles and Ewan (1982), a GE digestibility value of 83.4 % can be calculated. Chae and Lee (2002) reported GE digestibility values of 65.8 and 78.31 % for raw and extruded samples of RB, respectively. Brooks and Lumanta (1975a) reported a GE digestibility value of 66.0% in RB. The GE, DE, ME, and NE values in RB are presented in Table 1-2; the GE digestibility values in Table 1-3.

Protein and Amino Acids

Differences in crop quality, milling procedures, as well as hull adulteration make it difficult to assign specific values to the contents of nutrients and energy in RB. Warren and Farrell (1990a) reported an average crude protein (**CP**) value of 14.7% for full fat RB (**FFRB**) from different Australian – grown rice cultivars. Season of harvesting and variety were believed to be responsible for the variation in CP content which ranged from 13.4 to 17.3 %. Saunders (1990) reported CP values ranging from 12 to 16 % for RB, from 17 to 20 % for parboiled RB without calcium carbonate, and from 14 to 18 % for parboiled RB with the inclusion of 4 to 6 % calcium carbonate. Robles and Ewan (1982) reported a CP value of 13.1 %, which is similar to the value reported by Brooks and Lumanta (1975a), who reported a CP content of 13.4 %. Further, Resurreccion et al. (1979) reported a CP content of 14 % for RB and values ranging between 13.1 and 18.8 % for rice polish, which is often a component of RB. Other sources claim that "polish" can range in CP content from 11.8 to 13 %. In comparison, wheat bran, barley bran and oat bran may have values of 14.5 to 15.7 %, 11%, and 8.8 to 16.2 %, respectively (Juliano, 1985).

The amino acid profiles for RB reported by Warren and Farrell (1990a) are usually more balanced than those of other cereal grains (Farrell, 1994). Warren and Farrell (1990a) reported the amino acid contents in FFRB from three rice cultivars. The contents of the amino acids that are often first-or second- or third-limiting in diets for pigs, namely lysine, threonine and methionine ranged from 0.82 to 0.91 %, from 0.54 to 0.64 %, and from 0.24 to 0.43 %, respectively. Farrell (1994) stated that the amino acid profiles of RB from these Australian cultivars are very similar to those of 15 good quality

RB samples from Southeast Asia. Brooks and Lumanta (1975a) reported that the contents of lysine, methionine, threonine, and cystine in RB were 0.55, 0.44, 0.65 and 0.12 %, respectively. The contents of lysine, methionine, threonine, and tryptophan in RB were 0.57, 0.26, 0.48, and 0.14 %, respectively, as reported by NRC (1998). The CP and amino acid contents, reported in different literature sources, are presented in Table 1-2.

The digestibility of CP (fecal) and selected amino acids (ileal) are presented in Table 1-3. Sauer et al. (1989) reported an apparent fecal CP digestibility value of 57.4 %, while Chae and Lee (2002) reported values of 65.1 and 76.1 % for raw and extruded samples of RB, respectively. These values are in agreement with those reported by Brooks and Lumanta (1975a). The apparent ileal digestibilities of amino acids in RB in pigs vary considerably. The NRC (1998) reported values of 72, 74, 61, and 64 % for lysine, methionine, threonine, and tryptophan, respectively. In comparison, Rademacher et al. (1999) reported apparent ileal digestibility values of 55, 67, 50, and 66 % for lysine, methionine, threonine, and tryptophan, respectively. There are certain factors such as dietary fiber and fat content that may affect the apparent ileal amino acid and fecal CP digestibilities. With the equivalent of 16.8% NDF in a cornstarch-based soybean meal diet, there was no depression in the ileal amino acid digestibility values in young pigs (Li et al., 1994). However, in studies with growing-finishing pigs, there was a decrease in the fecal digestibility of CP as the dietary fiber content was increased (Sauer et al., 1980; Partridge et al., 1982). The increase in dietary fiber content is associated with a decrease in the content of readily digestible dietary components, and a greater proportion of the less digestible carbohydrates. Consequently, more nutrients escape enzymatic digestion

in the small intestine, and are subjected to bacterial fermentation in the large intestine (Just, 1982 a,b). Most of the nitrogenous substances absorbed in the large intestine do not contribute to the protein status of the pig because the majority of nitrogen (in the form of urea) is excreted in urine (Just et al., 1981). In the presence of sufficient availability of substrates as sources of energy for the microflora in the large intestine, there will be a decrease in the absorption of nitrogenous compounds and an increase in the assimilation of these in bacterial protein, resulting in a lower CP (and also GE) digestibility. This may be of some importance, as different samples of RB contain various levels of NDF. Also in young pigs, it appears that the apparent ileal digestibilities of most amino acids increase linearly with increasing dietary fat content (Li and Sauer, 1994). Similar results were reported when the dietary fat content was increased in diets for growing-finishing pigs (Imbeah and Sauer, 1991). Again, these findings may have relevance, depending on the level of inclusion of RB in the diet and its fat content.

Lipids

The crude fat (**CF**) contents in RB from different literature sources are presented in Table 1-2. Rice bran usually contains 16 to 22 % CF (Saunders, 1990). Chae and Lee (2002a) reported that FFRB contained 16.95 % CF, while defatted rice bran (**DFRB**) contained 1.25 % CF. A value of 2.1 % was reported for the CF content of a sample of DFRB by Sauer et al. (1989). Warren and Farrell (1990a) reported a range of 20.4 to 23.4 %. Brooks and Lumanta (1975a) reported a CF value of 14.8 % and a corresponding fecal digestibility of 91.4 %. They also reported an increase in CF digestibility with an increase of inclusion of RB in a basal diet. Chae and Lee (2002a)

reported a fecal CF digestibility of 81.3 and 85.2 % for a raw and extruded sample of RB, respectively.

Palmitic, oleic and linoleic fatty acids constitute 90 % of the total fatty acids in RB (Saunders, 1990). This is in agreement with values reported by Warren and Farrell (1990a); the contents of palmitic, oleic and linoleic fatty acids were found in amounts of 16, 42 and 39 % of extracted oil, respectively. These three fatty acids accounted for 96 % of the oil content.

Removal of bran and polishing of rice activates the lipolytic enzymes in bran, which leads to a release of free fatty acids and hydrolytic rancidity. This is likely the most important factor affecting the use of RB as a viable feedstuff. Shaheen et al. (1975) conducted a study on the effect of parboiling rice on the rate of lipid hydrolysis in rice bran. It was found that storage of untreated RB for more than one month resulted in considerable deterioration of the RB and its associated oil. However, if parboiled, the formation of free fatty acids was reduced, and the RB remained stable in storage for up to 10 months. Sapkota et al. (1982) also noted a reduction in free fatty acid content in RB during a 32-day storage period when it was subjected to temperatures ranging from 85 to 105°C.

Rancidity, the hydrolysis and oxidation of triglycerides to produce off-flavours, of dietary ingredients can affect the performance of animals by reducing feed intake due to poor palatability, as well as by affecting nutrient and energy digestibilities. Extrusion of RB resulted in higher digestibility values for GE and CP compared to values in either fresh or rancid raw RB (Chae and Lee, 2002a). These authors also reported that the digestibilities of some of the indispensable amino acids were lower in rancid than in fresh

RB. Pigs fed fresh RB had a slightly higher feed intake and significantly better feed conversion ratio and growth rates than those fed rancid RB. In terms of performance, these results agree with those of Hussein and Kratzer (1982) and Chae et al. (2002b) who reported poorer growth in chicks fed rancid compared to fresh RB.

Fiber

Values for NDF and ADF contents in RB were reported by the NRC (1998) to be 23.7% and 13.9%, respectively. Robles and Ewan (1982) reported values of 32.8 and 17.9 % for NDF and ADF, respectively. Examples of values for crude fiber contents ranged from 5 to 8 % (Fernandez and Jorgensen, 1986) and from 6.3 to 8.8 % (Resurreccion et al., 1979). The contents of fiber in RB, compiled from the literature, are presented in Table 1-2.

Several fiber-related factors influence the digestibilities of nutrients and energy. Usually, an increase in dietary fiber content is associated with a decrease in the digestibilities of nutrients and energy (Fernandez and Jorgensen, 1986). Sauer et al (1980) concluded that both ileal and fecal digestibilities of CP and amino acids decreased with increasing dietary crude fiber. This phenomenon, as stated previously, is likely due to the increased availability of undigested nutrients in the large intestine, which is responsible for increasing microbial activity in this region of the digestive tract. Sauer et al. (1980) also reported that the intake of 1 kg ground barley straw caused a 90 to 100 g increase in the fecal excretion of CP. In a review on the effect of fiber on digestibility in pigs, Fernandez and Jorgensen (1986) compiled the following fiber-related factors. 1. Source of fiber. Each percentage unit increase in crude fiber from RB (% of intake) resulted in a 2.2 and 2.1% decrease in digestibilities of CP and energy, respectively.

Crude protein and energy digestibilities were reduced by 1% for each percentage unit increase in NDF from RB. 2. Transit time. Although transit time is reduced with increasing fiber content, it is not believed to be a major factor for reducing digestibility (Ehle et al., 1982; Stanogias and Pearce, 1985). This is applicable to RB since different samples exhibit a wide range in fiber content. 3. Technical treatment. Processing procedures such as grinding and heat-treatment can alter the nutritive value of a feedstuff. Various types of rice-milling facilities exist throughout the world, which result in different proportions of hulls and straw in RB, which also may undergo further treatment to prolong its storage life and increase its nutritive value. 4. Adaptation. Reports on the effect of increasing dietary fiber content on anatomical changes in the pig, such as weight and size of organs associated with the digestive tract, are conflicting. 5. Feeding level. The digestibility of nutrients decreases as dietary fiber content increases, but the level of feeding can affect the magnitude. For example, Fernandez et al. (1986) reported no significant differences in digestibility when feedstuffs ranging from 0 (animal origin) to 43.5 % crude fiber were fed to sows at different levels (80, 100 and 120 %) of maintenance. In contrast, Cunningham et al. (1962) fed cellulose at 40% in grower diets, and reported a resulting decrease in digestibility. In terms of RB, this factor is important in that it may have consequences regarding the correct level of inclusion in diets for monogastric animals. 6. Age and live weight. It is acknowledged that as pigs grow, their digestive potential increases. As was reviewed by Fernandez and Jorgensen (1986), there is ample evidence that the GE, CP and fiber digestibilities are higher for sows than for growing pigs.

Minerals

The content of minerals in RB is quite variable. Growing conditions, cultivar of rice, and milling procedures are all responsible for the variation. For example, RB produced in the U.S. contains no appreciable amounts of hulls; therefore, the presence of silica would be much lower than in RB from other sources. Chae and Lee (2002a) used a sample of FFRB in their experiment, which contained 0.32 % Ca and 1.40 % P and had a crude ash content of 8.75 %. Another sample of DFRB contained 0.36 % Ca and 1.73 % P and 13.18 % crude ash. Saunders (1990) reported a range of ash content from 7 to 10 %; values reported in other publications are within this range, or very close (Warren and Farrell, 1990a). In a review of the properties of RB, Saunders (1990) reported the concentrations of several minerals. Calcium ranged from 0.014 to 0.131 % and P from 1.48 to 2.87 %. The contents of Na, Al, Zn, Mn, Fe, Cl, Si, Mg, and K were (ranged from): 0 to 0.029, 0.0053 to 0.037, 0.008, 0.011 to 0.088, 0.019 to 0.053, 0.051 to 0.097, 0.17 to 1.63, 0.865 to 1.23, and 1.365 to 2.390 %, respectively. It is interesting to note that 80 % of the iron in the rice kernel is contained in the bran. The calcium, P, Mg, Zn and Fe contents in FFRB samples were also determined by Warren and Farrell (1990a). The Ca, P and Mg contents ranged from 0.027 to 0.051 %, 1.623 to 1.811 %, and 0.607 to 0.771 %, respectively. The Zn and Fe concentrations ranged from 0.0044 to 0.0054 % and from 0.0038 to 0.0048 %, respectively. The Ca and P contents in RB reported in the literature are presented in Table 1-2.

Approximately 90 % of P in RB is present in the form of phytate (Nelson et al., 1968; Juliano, 1985). Most of the P contained in phytate is not available to monogastric animals. In fact, only 17.6 % of P is available (Corley et al., 1980). Also, phytate may interfere with the availability of other minerals via chelation, for example, with calcium.

The phytate content in FFRB (4.21 to 4.37 %) does not vary as much as it does in DFRB varieties (2.40 to 4.60 %) (Warren and Farrell, 1990a). Phytate-P, as well as most other minerals, is concentrated mainly in the aleurone and pericarp layers of the bran (O'Dell et al., 1972).

B. Animal Performance

Several studies, mostly with poultry, have been carried out to determine the optimum inclusion rates of RB in both poultry and swine diets (Majun and Payne, 1977; Srichai and Balnave, 1981; Tangendjaja et al.; 1988 Warren and Farrell, 1990c). Warren and Farrell (1990c) reported that a layer diet containing 25 % DFRB gave a lower egg production than either a commercial diet or one containing the equivalent amount of wheat bran. The ADG of broilers was also reduced with increasing amounts of RB in the diet when raw, autoclaved or dry-heated RB was included to replace corn at levels of 75 and 100 % levels in the diet (Deolankar and Singh, 1979). Another purpose of the same study was to determine whether the trypsin inhibitor activity in RB was responsible for the depression in growth. However, the diets without RB also contained trypsin inhibitor activity, and therefore, the inhibitor was not entirely responsible for the reduction in growth. Rice bran that was autoclaved yielded poorer growth rates than either dry-heated or raw RB possibly due to the destruction of some essential amino acids during autoclaving. Conversely, Kratzer et al. (1974) reported a significant improvement in ADG of chicks when RB was either autoclaved or steamed. When the level of inclusion of RB in the diet was increased to 60 %, there was a marked decrease in ADG (68 % of control-fed birds), which perhaps indicates the presence of a growth-depressing factor in RB. This suspicion was further supported by the fact that 60 % inclusion of wheat bran

in the diet resulted in no depression of growth, even though wheat bran contains less metabolizable energy than RB. Bersch et al. (1989) also reported that the dietary inclusion of autoclaved RB (121 °C, 15 min) compared to raw or HCl-stabilized RB resulted in a higher ADG. Nevertheless, it is difficult to draw conclusions on the optimum dietary level of inclusion of RB. Rice bran that was stabilized by extrusion cooking at 130°C, and included in a chick diet at a level of 60 %, produced gains close to chicks fed a commercial diet that contained 60 % corn (Sayre et al., 1987). The feed:gain ratio was also improved.

The inclusion of RB beyond a certain point also seems to impair growth in swine. Rice bran subjected to cold water, hot water, cooking or ensiling treatments, and included at 40 % in a corn-soybean meal diet gave a significantly lower ADG in pigs than those fed the corn-soybean meal diet (Tangendjaja et al., 1988). The inclusion of untreated RB yielded a better ADG than any of the treated RB; the ensiling treatment of RB gave the lowest ADG which was reduced by 20 %. Furthermore, there were no differences in GE and CP digestibilities between cold water, hot water, cooking, or ensiling treatments, but the GE digestibilities were higher in diets containing extruded and pelleted RB than untreated RB. The same authors, in a second trial, compared extruded and pelleted RB and reported that both these treatments improved feed : gain ratios by about 10 % compared to the corn-based diet. Serra et al. (1983) reported similar results in performance with the inclusion of pelleted RB. Tangendjaja et al. (1988) also reported that RB may be included at a level of 22 % in grower and finisher swine diets without affecting performance. These results are in agreement with those of Warren and Farrell (1990b) who suggested that 20 % inclusion may be close to the upper limit for optimum

incorporation of DFRB into a swine diet. Pigs consuming diets containing 20 and 30 % DFRB had a higher ADG than those consuming the commercial diet, but the differences were not significant. Therefore, diets containing up to 30 % DFRB gave the same performance as the commercial diet in terms of ADG or feed conversion efficiency. Another study showed that RB can replace up to 50% of corn in a swine diet without adversely affecting performance or carcass characteristics (Baird et al., 1975). These researchers replaced corn with RB at levels of 0, 25, 50, 75 and 100% in a corn-based diet. In the same order for the diets, the ADG (kg) were 0.73, 0.73, 0.69, 0.65, and 0.54 kg, respectively. The feed:gain ratios were 3.34, 3.26, 3.46, 3.34, and 3.21, respectively. Therefore, if more than 50 % corn is replaced by RB, there will be significant decrease in ADG. In another study, Brooks and Lumanta (1975b) included RB at levels of 0, 40, 60 and 80 %. When the inclusion of RB exceeded 40 %, there was a significant decrease in ADG. In the same order for the diets, the ADG (kg) were 0.74, 0.75, 0.69, and 0.64, respectively. The feed:gain ratios were 2.95, 3.09, 3.04, and 3.22, respectively. It was also reported that with increasing levels of RB inclusion, there was a decrease in backfat thickness and increase in loin eye area.

C. Experimental Methods

The objective of the present studies was to determine the chemical and amino acid composition and the digestibility values of GE, CP, and, of primary importance, amino acids in different sources of RB. The digestibility of GE should be determined with the fecal analysis method and is defined as the difference between the amount of energy in the diet and in feces, divided by the amount in the diet. The digestibilities of amino acids should be determined with the ileal rather than fecal analysis method. Ileal amino acid

digestibility is defined as the difference between the amount of amino acids in the diet and in ileal digesta, divided by the amount in the diet (Low, 1982; Sauer and Ozimek, 1986). Several studies have indicated that a more accurate estimation of amino acid digestibility can be obtained with the ileal analysis method (Low, 1982; Sauer and Ozimek, 1986 and Sibbald, 1987). However, even with the ileal analysis method, there can be significant variation in ileal digestibility values between samples of the same feedstuff due to variety, level of fertilization, environmental conditions, processing, and variation in the levels of amino acids in the feedstuffs (Sauer and Ozimek, 1986).

Ileal and Fecal Digestibility

The ileal analysis method is preferred for determining amino acid digestibility values. Amino acid digestibility values are not well represented by fecal digestibility values because feces contains a mixture of amino acids from undigested dietary residues, endogenous secretions and bacteria (Low, 1982). Protein that is not digested in the stomach and small intestine passes into the large intestine. Some of the protein is voided in feces as such; the remainder is fermented by the microflora in the large intestine. The process of fermentation includes deamination, decarboxylation, and desulfhydration. During this process there will be production of nitrogen-containing compounds such as ammonia (in particular), and amines, and de novo microbial protein synthesis. The ammonia that is produced will be absorbed (and excreted in the form of urea in the urine) or used for the synthesis of microbial protein. The level of de novo microbial protein synthesis depends on the amount and nature of carbohydrate that enters the large intestine. The events that occur as a result of microbial fermentation in the large intestine do not affect nitrogen balance in the pig. Nitrogen balance was not affected when amino

acids were infused into the large intestine of pigs fed a protein-free diet (Zebrowska, 1973). Up to 76 % of the nitrogen in feces can be attributed to bacterial sources (Mason, 1984). However, the digestibility of GE should be determined with the fecal analysis method, as fermentation in the large intestine produces heat and VFA, which are of use to the pig. The VFA are the principle products of bacterial fermentation in the large intestine in the pig, and comprise approximately 98 % of the organic acids (Yen, 2001). Acetic acid is the most common VFA, followed by propionic and butyric acid. These VFA are absorbed and used in intermediary metabolism in the intestinal and peripheral tissues and the liver.

Apparent and True Digestibility

Low (1982) defines true amino acid digestibility as similar to apparent digestibility except that the amount of endogenous amino acids (from sources such as sloughed cells, saliva, and bile and pancreatic secretions) in feces or ileal digesta are subtracted from the total amount in the feces or digesta. Regression analysis, ^{15}N-isotope dilution techniques or the use of protein-free diets are the methods used to measure the amount of endogenous amino acids. It has been shown that the use of a protein-free diet is not the best approach, as it underestimates amino acid digestibility values, and thus the true digestibility (de Lange et al., 1989). These secretions are influenced by the level of intake as well as the crude fiber content of the diet. De Lange et al. (1989) reported that the addition of cellulose tended to increase the amount of endogenous protein and most of the amino acids in ileal digesta. Some researchers believe that the ^{15}N-isotope dilution techniques are the most accurate methods (Leterme et al., 1991), as non-digested dietary protein can be distinguished from endogenous protein.

Digestibility Techniques

Two techniques are available to determine the apparent ileal or fecal digestibilities of nutrients and energy in a diet: the total collection and the indicator technique. Both techniques require an assay diet to be fed, and ileal digesta and feces are collected. In this experiment, the indicator method was chosen for reasons of accuracy and convenience. With the total collection technique, the entire production of digesta and/or feces, and feed consumption must be accurately measured. These measurements are subject to error, even when one takes extreme care. The indicator technique, however, avoids some of these shortcomings by the incorporation of a digestibility marker into the diet (e.g. chromic oxide). The larger the amount of ileal digesta and feces that are collected, the more accurate the determination of the digestibility values.

Fan and Sauer (1995a,b) described three methods for determining the nutrient and energy digestibility values in a feedstuff, namely the direct, the difference, and the regression method. The direct method is very simple in that a diet is formulated in which the assay ingredient provides the only source of a particular nutrient. Therefore, the nutrient digestibility value for this assay ingredient is equivalent to the dietary nutrient digestibility obtained via the indicator technique. Fan and Sauer (1995a) showed that the direct method is not suitable for obtaining apparent digestibility values for feedstuffs that are low in protein; they stated that the reason for this is the relatively high endogenous amino acid contribution when a low protein diet is fed. In a study by Fan et al. (1995), it was found that the levels of endogenous amino acids, expressed as g/kg dry matter intake, are constant at different dietary amino acid levels. Therefore, with respect to apparent ileal amino acid digestibility values, if the intake of amino acids decrease, and

endogenous amino acid levels remain constant, then endogenous amino acids will account for a higher proportion of amino acids in ileal digesta. However, the contributions of endogenous amino acids, as a percentage of dietary contents, decrease curvilinearly with increasing dietary contents. The direct method is, however, suited to determine the digestibility values in feedstuffs high in protein, for example soybean meal and canola meal. For the studies in this thesis, the sole source of nutrients in the basal diet (and similarly the basal feed ingredient) did not originate from a single ingredient, but a combination of corn and soybean meal. The direct method was used to determine the digestibility values of corn and soybean meal.

The difference method for the determination of the digestibility value of a nutrient or energy involves the formulation of both a basal and an assay diet. The basal diet contains the basal feed ingredient which provides the only nutrients in the diet. The assay diets consist of a mixture of the basal feed ingredient and the assay feed ingredient. The nutrient digestibilities in the assay feed ingredients can then be determined by difference. It is assumed that there are no interactions between the basal and assay feed ingredient. Fan and Sauer (1995a) showed that the difference method should be the choice for determining digestibility values in feedstuffs that are relatively low in protein and/or relatively high in fiber. The difference method, therefore, was used to determine the energy and ileal amino acid digestibility values in RB.

The regression method evaluates the basal and assay feed ingredients simultaneously. Both the basal and assay feed ingredient provide the only nutrients. A number of assay diets are formulated, in which the assay and basal feed ingredients are graded. It is assumed that there is no interaction between the basal and assay feed

ingredient. By use of regression analysis, one can calculate the nutrient digestibility values of the assay feed ingredients (and also of the basal feed ingredient). This method can also be used to determine the amino acid digestibility values in RB, but is not practical if information is needed on more than one source of RB. If this were the case, several graded diets would have to be formulated for each particular source of RB, which would be inconvenient in terms of cost and experimental size.

D. Objective of Thesis

The optimum inclusion rate of a feedstuff in diets for monogastric animals is often confounded by the variability of nutrient and energy contents, as well as the digestibilities of these between different sources of the same feedstuff. Several factors contribute to the variation, such as the cultivar of the plant, climatic conditions, and different milling processes. There is a scarcity of information in the literature concerning the contents and apparent ileal digestibilities of gross energy and amino acids in different sources of rice bran. Furthermore, no studies have been carried out to determine which factors, i.e. NDF, CF, CP, and ash contents in RB, affect amino acid digestibility, or the extent to which these factors are responsible for the variation in digestibility. In addition, no research has been carried out to determine the contents of CP and amino acids associated with NDF, their contributions to the total CP and amino acid content in RB, or their ileal digestibilities. These are important considerations, as there may be considerable levels of amino acids sequestered by the NDF in RB.

In Chapter 2, studies were carried out, therefore, to determine the chemical composition and apparent ileal digestibilities of CP and amino acids in different samples of RB. As well, simple linear relationships were established between the apparent ileal digestibilities of amino acids and the contents of NDF, CF, CP, and ash in the different samples of RB.

In Chapter 3, the apparent ileal digestibilities of amino acids associated with the NDF fraction of RB were determined. Simple linear relationships were then established between the apparent ileal digestibilities of amino acids and the content of CP associated with the NDF fraction in the different samples of RB.

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Table 1-1. Chemical composition (%) of rice hulls[a]

Item	Rice hull
Total digestible nutrients	9.3-9.5
Crude protein	1.9-3.0
Crude fat	0.3-0.8
Available carbohydrates	26.5-29.8
Crude fiber	34.5-45.9
Neutral detergent fiber	66-74
Acid detergent fiber	58-62
Lignin	9-20
Cellulose	28-36
Crude ash	13.2-21.0
Silica	18.8-22.3
Calcium	0.06-0.13
Phosphorous	0.03-0.07

[a]Juliano (1985)

Table 1-2. Chemical composition (%) of rice bran

Item	Rice bran			
	Reference 1[a]	Reference 2[b]	Reference 3[c]	Reference 4[d]
Dry matter	90		91.8	
Energy, kcal/kg				
Gross energy		4,700		4,650
Digestible energy	3,100			3,880
Metabolizable energy	2,850			3,710
Net energy	2,040			
Crude fat	13.0	14.8	20.4-23.4	10.8
Crude fiber		12.4		11.7
Neutral detergent fiber	23.7		20.1-29.2	32.8
Acid detergent fiber	13.9		9.4-14.0	17.9
Lignin			2.9-5.3	4.5
Crude protein	13.3	13.4	14.1-18.2	13.1
Amino acids				
Lysine	0.57	0.55	0.82-0.91	
Methionine	0.26	0.44	0.24-0.43	
Threonine	0.48		0.54-0.64	
Tryptophan	0.14			
Calcium	0.07	0.12	0.027-0.051	
Phosphorous	1.61	1.34	1.62-1.81	

[a]NRC (1998)
[b]Brooks and Lumanta (1975a)
[c] Warren and Farrell (1990a)
[d]Robles and Ewan (1982)

Table 1-3. Apparent fecal digestibilities (%) of energy and crude protein and apparent ileal digestibilities of selected amino acids in rice bran

Item	Rice bran digestibility			
	Reference 1[a]	Reference 2[b]	Reference 3[c]	Reference 4[d]
Energy			65.8	66.0
Crude protein			65.1	69.7
Amino acids				
Lysine	72	55		
Tryptophan	64	66		
Threonine	61	50		
Methionine	74	67		
Cystine	66	56[e]		

[a]NRC (1998)
[b]Rademacher et al. (1999)
[c]Chae and Lee (2002a)
[d]Brooks and Lumanta (1975a)
[e]Methionine and cystine together

CHAPTER 2

CHEMICAL COMPOSITION AND ENERGY, PROTEIN, AND AMINO ACID DIGESTIBILITY IN DIFFERENT SOURCES OF RICE BRAN FOR GROWING PIGS

A. Introduction

Rice bran (**RB**) is removed during the process of milling of rice to produce white rice for human consumption. The fractions of rice that are collectively referred to as RB include the pericarp, seed coat, nucellus and aleurone, as well as most of the germ. Studies have been conducted to examine the feasibility of using RB as an ingredient in poultry (Kratzer et al., 1974; Majun and Payne, 1977; Sayre et al., 1987; Warren and Farrell, 1990b) and in swine diets (Baird et al., 1975; Brooks and Lumanta, 1975a,b; Serra et al., 1983; Tangendjaja et al., 1988; Warren and Farrell, 1990b).

Rice bran originating from different rice cultivars and from rice grown under different environmental conditions may differ in chemical composition. Different milling operations may also affect its chemical composition. Typically, RB contains 12 to 16 % CP and about 23 % NDF. Warren and Farrell (1990 b) have shown that RB included in a grower pig diet at up to 300 g/kg resulted in a satisfactory growth rate. Other studies (Majun and Payne, 1977; Serra et al., 1983) have shown that the nutritive value of RB can be improved by different processing methods. Because of the large quantity of RB produced throughout the world, it is important to determine not only the nutritive value, but also the variation, in samples of RB of different origin. The objective of this study was to determine the proximate and amino acid composition, as well as the GE digestibility and apparent ileal digestibilities of amino acids in five samples of RB.

B. Materials and Methods

Animals and Diets

Six barrows, with an average initial BW of 36.5 kg, were obtained from the University of Alberta Swine Research and Technology Centre. The animals were individually confined in stainless steel metabolic crates in a temperature-controlled facility (20 to 22°C). Following a 7-d adaptation period to the metabolic crates, the barrows were fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer et al. (1983). The cannulas were modified according to de Lange et al. (1989). A detailed description of pre- and post-operative care was previously presented by Li et al. (1994). A 12-d recuperation period followed, and during this time, the barrows were fed an 18 % CP diet (Table 2-1, Diet F).

After recuperation, the barrows were fed one of six experimental diets (Table 1) according to a 6 × 6 Latin square design. They were given two meals of equal amounts each day, at 0800 and 2000, with the daily dietary allowance equivalent to 4 % (wt/wt) of the individual BW determined at the beginning of each experimental period. Water was available at all times from a low-pressure drinking nipple. The average BW of the barrows at the conclusion of the experiment was 98.6 kg.

Six diets were formulated (Table 2-1) to contain 18 % CP (% N × 6.25, as-fed basis). Diets A, B, C, D and E contained 53.8 % corn, 19.9 % soybean meal (**SBM**) and 23.1 % RB. Five different sources of RB were obtained. Rice bran 1: Canadian source of RB; RB 2: Riceland Foods Inc., Stuttgart, AR, 72160; RB 3: Agribrands International, Italy; RB 4: North Luzon Region, Phillipines; and RB 5: Agribrands/Tradico, Greenville Feed Facility, Greenville, MS., 38701. Diet F contained 71.03 % corn and 26.3 % SBM.

Vitamins and minerals were supplemented to meet or exceed NRC (1998) standards, and chromic oxide was included in the diets at .3 % as the digestibility marker. The diets were fed in a mash form.

The duration of each experimental period was 11 d. The collection of feces was initiated at 0800 on d 8 of each experimental period and continued for 48 h. Ileal digesta were collected for a total of 24 h; from 0800 to 2000 on d 9 and from 2000 on d 10 to 0800 on d 11. The procedure for digesta collection was adapted from Li et al. (1994). Feces and digesta were frozen at -28°C immediately after collection, and the samples were pooled within barrows and periods. The barrows used in this experiment were cared for in accordance with the guidelines established by CCAC (1993) and approved by the Faculty of Agriculture, Forestry and Home Economics Animal Care Committee of the University of Alberta.

Chemical Analyses

Prior to analyses, samples of ingredients, diets, oven-dried feces and freeze-dried digesta were ground through a 1-mm mesh screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA). Analyses for DM, crude fat (CF) and ash were carried out according to AOAC (2000). Gross energy was determined using a Parr 1241 Adiabatic Bomb Calorimeter (Parr Instruments, Moline, IL). Crude protein (% N × 6.25) was determined with a Leco FP-428 Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). The analysis of NDF in the diets and ingredients was carried out using a fiber bag apparatus (Ankom Co., Fairport, NY). Approximately .5 g of each sample was measured into a filter bag, which was then heat-sealed. Each sample was submerged overnight in 100 ml NDF solution with 100 µl heat-stable α-amylase (EC 3.2.1.1; Sigma Chemical

Company, St. Louis, MO). The bags were placed on a bag suspender in 100 ml of NDF solution at 97 to 99°C. After agitating for 70 min., the bags were removed and immediately rinsed in water (80 to 99°C) twice, followed by several more rinses with the addition of 100 µl of heat-stable α-amylase. This procedure was repeated until no starch remained as determined with the iodine test. The bags were then soaked for 2 to 3 min. in acetone, air-dried, and placed in an oven at 110°C overnight. Chromic oxide was measured according to Fenton and Fenton (1979).

Amino acid analyses were carried out at Degussa AG, Hanau-Wolfgang, F.R.G. Prior to analyses, samples were ground through a 0.5-mm mesh screen. Analyses for tyrosine were not performed. For amino acid analyses, except for tryptophan, the following procedure was used. A sample containing approximately 10 mg nitrogen (the total quantity of sample may vary from 100 to 800 mg) was placed into a 50 ml flask. Then, 5 ml of cold performic acid was added, and the solution was stirred with a magnetic rod for 15 min. while in an ice bath. The flask was then closed and allowed to react for 16 h at 0°C to yield methionine sulfone and cysteic acid. Following, excess performic acid was destroyed by adding about 0.84 g sodium disulphite, while stirring for 30 min. in an ice bath and exhausting any sulphur dioxide into a fume hood. Thereafter, hydrolysis was initiated by adding 25 ml hydrochloric acid-phenol reagent into the flask and placing it in an oven at 110°C for 24 h.

After hydrolysis, the flasks were cooled and 15 ml norleucine standard solution was pipetted into each flask. After mixing, the hydrolysate was transferred to a 250 ml beaker, to which 125 ml citrate buffer and 19 ml sodium hydroxide solution were added. Sodium hydroxide solution (1 mol/L) was used to adjust the pH to 2.20. A portion of the

resulting solution was transferred to a 50 ml flask, and frozen until analysis. This solution was then applied to the column of the amino acid analyzer. The amino acid analyzer was a Biochrom 20 with a two-channel A/D converter card. Amino acid chromatograms were simultaneously detected at 570 and 440 nm. The EZ Chrom™ integration software (Scientific Software Inc., USA) was used for integration of the chromatograms.

For the oxidation mixture, an 88 % phenolic formic acid solution was prepared by mixing 889 g formic acid with 111 g distilled or deionized water and approximately 5 g phenol. The final oxidation mixture was prepared by mixing 10 ml 30 % hydrogen peroxide with 90 ml of the phenolic-formic acid solution in a 100 ml Erlenmeyer flask. Thereafter, the flask was sealed and allowed to stand for 1 h at 20 to 30°C to ensure the formation of performic acid. The hydrochloric acid-phenol hydrolysis reagent (6 M HCl) was formulated by dissolving 5 g phenol in 2,500 ml water in a 5,000 ml flask. Then, 2,500 ml of 37 % hydrochloric acid was added and mixed. The norleucine standard was prepared by dissolving 2 g norleucine in 500 ml of 1 mol/L hydrochloric acid, and topped up with water in a 5,000 ml volumetric flask. The citrate buffer required 98 g sodium citrate and 5 g phenol to be dissolved in 4,000 ml water in a 5,000 ml beaker. Then, 25 ml thiodiglycol and 80 ml 37 % hydrochloric acid were added, and diluted with 800 ml water. The pH was adjusted to 2.2 with hydrochloric acid, and the mixture was transferred to a 5,000 ml volumetric flask, which was topped up with water. The sodium hydroxide solution (7.5 mol/L) was prepared by weighing 937 g of 32 % sodium hydroxide solution into a 1,000 ml volumetric flask while stirring, and then topped up with water.

The determination for tryptophan was carried out as follows. A sample of the amount previously mentioned was weighed into a 50 ml flask along with 8.4 g barium hydroxide and 12 ml water. The sample was stirred, and placed in a wire basket. Next, 3 L of water was poured into an autoclave, which was preset at 110°C, and the sample was autoclaved for 20 h at a pressure of approximately 0.4 bar. Following hydrolysis, the autoclave was allowed to cool to 90°C before removing the sample. Approximately 30 ml water and 2 ml internal standard were added to the hot hydrolysate, which was then cooled in an ice-water bath while stirring for 15 min. In the following order, 5 ml phosphoric acid and 5 ml hydrochloric acid (6 mol/L) were added, and the pH was adjusted to 3.0 with 1 mol/L hydrochloric acid. The mixture was diluted to about 100 ml with water. A portion of the hydrolysate was filtered into a beaker, and the filtrate was then diluted 1:5 with 30 % methanol. This solution was then passed through a 0.45 µm membrane filter and frozen. The methanol, tryptophan and internal standard were then separated by reversed-phase chromatography RP-18 on an HPLC column.

The internal standard solution (2.5 mmol/L) was prepared by dissolving 273 mg α-methyl-DL-tryptophan in 500 ml 0.1 mol/L hydrochloric acid, and refrigerated. The phosphoric acid solution (0.5 mol/L) was prepared by diluting 58 ml *ortho*-phosphoric acid with 1000 ml water. The methanol solution was made by simply placing 300 ml methanol into a 1000 ml volumetric flask and then topping up with water.

Analyses of ingredients and diets were performed in triplicate, and digesta and feces in duplicate.

Calculations and Statistical Analysis

The apparent ileal DM, CP and amino acid digestibilities as well as the fecal digestibility of GE of the experimental diets were determined using the indicator technique, which is represented by Equation 1.

$$D_D = 100\% - [(I_D \times A_F) / (I_F \times A_D)] \times 100\% \quad \text{(Equation1)}$$

Where D_D: apparent digestibility value of a nutrient in the assay diet (%); I_D: indicator concentration in the assay diet (g kg^{-1}); A_F: nutrient concentration in ileal digesta or feces (g kg^{-1}); I_F: indicator concentration in ileal digesta or feces (g kg^{-1}); A_D: nutrient concentration in the assay diet (g kg^{-1}).

In order to calculate the apparent digestibilities of DM, CP, amino acids and GE in the samples of RB, it is necessary to know the digestibilities of these in the basal feed ingredient, which was a mixture of corn and SBM. Since these ingredients are the sole source of nutrients, the digestibilities of these in the assay feed ingredients are equivalent to the digestibility values for the diet.

Lastly, the apparent nutrient digestibilities of the assay feed ingredients were calculated using the difference method, according to Equation 2. An assay diet, which contains a mixture of the basal ingredients (corn and SBM) as well as the assay ingredient (RB), and a basal diet (corn and SBM) are used to determine nutrient digestibility.

$$D_A = (D_D - D_B \times S_B) / S_A \quad \text{(Equation 2)}$$

Where D_A: apparent digestibility of a nutrient in the assay feed ingredient (%); D_D: apparent digestibility of a nutrient in the assay diet (%); D_B: apparent digestibility of a nutrient in the basal feed ingredient (%); S_B: contribution level (%) of a nutrient from the

basal feed ingredient to the assay diet; S_A: contribution level (%) of a nutrient from the assay feed ingredient to the assay diet.

Statistical analysis of the digestibility values of the experimental diets and RB was performed as a 6 × 6 Latin square using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Means of the dietary treatments, experimental periods and pigs were compared using the Student-Newman Keul's multiple range test. Relationships between the NDF, CF, CP, and ash contents and apparent ileal digestibility values of CP and amino acids in RB were determined using the simple linear regression procedure of SAS (SAS Inst. Inc., Cary, NC).

C. Results and Discussion

The pigs remained healthy and consumed their daily allowances throughout the experiment.

The formulation of the experimental diets is presented in Table 2-1. The chemical and amino acid composition of corn, SBM, and the RB samples are presented in Table 2-2. The values for corn and SBM were in the range of values reported by NRC (1998). The content of GE was highest in RB 3, followed by RB 5, 1, 4, and 2. The GE contents ranged from 4,476 to 5,677 kcal kg^{-1}. Robles and Ewan (1982) and Brooks and Lumanta (1975a) reported GE contents in RB of 4,650 and 4,700 kcal kg^{-1}, respectively. The different GE contents in the RB samples reflect differences in their CF contents. There was considerable variation in the contents of CF, which ranged from 4.1 (RB 2) to 24.4 % (RB 3). Rice bran 2 was a sample of RB that was defatted. The origin of RB 4, which also has a low CF content, namely 5.4 %, will be discussed later.

The NDF contents in the samples of RB ranged from 2.1 % in RB 4 to 34.3 % in RB 3 (Table 2-2). Warren and Farrell (1990a) reported values ranging from 20.1 to 29.2 %, with an average content of 25.6 %. The very low content of NDF in RB 4 (2.1 %) can be attributed to the fact that this particular sample consisted mainly of "polish", which is largely made up of starchy endosperm removed from the rice kernel during processing. This sample can be considered an atypical source of RB. The ash content of the RB samples ranged between 4.8 and 21.0 % and was lowest in RB4, the atypical source of RB, and highest in RB 1. Pomeranz and Ory (1982) reported values for ash content, which ranged from 9.2 to 11.5 % and from 6.1 to 8.5 % for RB and "polish", respectively. The most abundant minerals in RB are phosphorus (1.5 to 2.9 %), potassium (1.4 to 2.4 %), silicon (0.17 to 1.63 %), magnesium (0.9 to 1.2 %), and calcium (0.014 to 0.131%) (Saunders, 1990).

The CP content in the RB samples ranged from 13.1 % in RB 4 to 20.3 % in RB 2 (Table 2-2). Warren and Farrell (1990a) reported values ranging from 13.4 to 17.3 % CP. As was mentioned previously, RB 4 consisted largely of starchy endosperm, and therefore has a relatively low CP content. The most abundant indispensable amino acids in RB were arginine, ranging from 0.99 to 1.73 % and leucine, ranging from 0.94 to 1.38 %. The least abundant indispensable amino acids were histidine, ranging from 0.34 to 0.61 %, methionine, ranging from 0.30 to 0.40%, and tryptophan, ranging from 0.17 to 0.28 %. Of the dispensable amino acids, the levels of glutamic acid and aspartic acid were highest, while the level of cystine (actually a semi-dispensable amino acid) was lowest. The amino acid contents in the RB samples were in the range of those reported by Brooks and Lumanta (1975a) but slightly higher than those reported by NRC (1998).

With increasing CP content among the RB samples, there was an increase in the amino acid content. This relationship is presented in Table 2-3, and was significant ($P < 0.05$) for all amino acids.

The chemical and amino acid composition of the experimental diets is presented in Table 2-4. The analyzed values of the experimental diets were very close to the values calculated from the analyzed values of the ingredients. The differences in the parameters that were analyzed in the experimental diets reflected the differences in the contents in the different RB samples that were included.

As is generally accepted, the digestibility of GE should be determined with the fecal analysis method while amino acid digestibilities should be determined with the ileal analysis method (e.g. Sauer and Ozimek, 1986). The apparent ileal DM, CP, and amino acid as well as GE digestibilities of the experimental diets are presented in Table 2-5. Among the RB-containing diets, the digestibility of GE was highest ($P < 0.05$) for diet D which contained RB 4, which has the lowest NDF content of the RB samples. There were no differences ($P > 0.05$) among the other RB-containing diets, of which the NDF contents ranged from 11.7 to 14.8 %. Robles and Ewan (1982) reported that the digestibility of GE decreased linearly with incremental additions of RB to a diet.

The ileal CP digestibilities of the RB-containing diets ranged from 64.8 to 72.0 % and was lowest ($P < 0.05$) for diet B. The apparent ileal digestibilities of the amino acids were usually also lowest ($P < 0.05$) in diet B and highest in diet D. The ileal CP digestibilities were lower than the respective amino acid digestibilities in the experimental diets. This results from the fact that a considerable proportion of CP in ileal digesta is present in the form of non-amino acid nitrogen. In studies with pigs fed

protein-free diets, Sauer (1976) and Landin (1992), respectively, reported that 27.8 to 44.5 and 41.7 % of nitrogen was present in the form of non-amino acid nitrogen. In a study with pigs fed a milk powder diet, about 85 % of nitrogen in ileal digesta was non-amino acid nitrogen (Dierick et al., 1986). Non-amino acid nitrogen includes nitrogen from amino sugars (glucosamine and galactosamine) (Landin, 1992) and likely ammonia and urea (Mosenthin, 1987).

The apparent ileal digestibilities of DM, CP, and amino acids as well as fecal GE digestibilities of the RB samples are presented in Table 2-6. For reasons of the relatively low CP and high NDF contents of RB, the ileal CP and amino acid digestibilities were determined according to the difference method which should be the method of choice (or regression method) for these types of ingredients (Fan and Sauer, 1995). The GE digestibility was highest for RB 4 (86.9 %), which has the lowest NDF content among the RB samples. The GE digestibilities ranged from 60.5 to 65.8 % for the other RB samples of which the NDF contents ranged from 25.5 to 34.3 %. Brooks and Lumanta (1975a) reported a value of 66 % for the digestibility of GE in RB.

There was considerable variation in the amino acid digestibilities among the RB samples (Table 2-6). For the more important amino acids in practical diet formulation (i.e. amino acids that are often first- or second-limiting), the apparent ileal digestibilities of lysine ranged from 62.6 to 82.2 %, of methionine from 61.4 to 76.0 %, of threonine from 58.4 to 82.8 %, and of tryptophan from 58.8 to 82.6 %. Rademacher et al. (1999) reported apparent ileal digestibilities of 55, 67, 50, and 66 % for lysine, methionine, threonine, and tryptophan, respectively (n = 1). Values reported by NRC (1998) were 72, 74, 61, and 64 % for lysine, methionine, threonine, and tryptophan, respectively. The

ileal CP and amino acid digestibilities were lowest in RB 2, a defatted source of RB. Warren and Farrell (1991), in studies with growing chickens, using the slaughter technique, also reported lower ileal amino acid digestibility values in DFRB than in FFRB. The digestibilities of lysine, methionine, and threonine were 54, 45, and 51 % in DFRB. In the same order for the amino acids, the digestibility values were 71, 71, and 66 % in FFRB. Depending on the processing conditions under which RB is defatted, there may be a negative effect on the digestibility of amino acids. Protein denaturation as a result of heat stabilization can impair the extractability of protein, and significantly reduce the content of lysine ($P < 0.05$) in RB (Gnanasambandam and Hettiarchchy, 1995). Lipase activity in RB will initiate the hydrolysis of lipids, leading to the formation of free fatty acids and subsequent rancidity, if the bran is not immediately defatted (Juliano, 1985). Solvent extraction using hexane is the common method of defatting RB. Prior to, or following solvent extraction, heat is applied to inactivate lipase. The RB is prepurified, steamed (precooked at 95 to 105°C), and dried, and then placed in an extractor into which hexane is pumped. The solvent mixes thoroughly with the bran, extracting the oil, and then the miscella (the solvent and oil mixture) is passed into an evaporator for desolventizing. Following solvent extraction, 0.5 to 2.5 % residual oil can remain in defatted RB, which can again be subjected to steaming for 5 to 10 min. at 93 to 104°C to inactivate lipase activity (Young et al., 1991).

The values for amino acid digestibility were highest in RB 4, which has the lowest NDF content, in RB 5, an extruded source of RB, and RB 1. Extrusion is a form of either added-moisture-, or retained-moisture wet-heat stabilization, and is used to achieve lipase and typsin inhibitor inactivation (Deolankar and Singh, 1979), and

sterilization of RB without the necessity of oil extraction. Lipase inactivation can be achieved in 3 to 5 min. at 100 to 110°C. A detailed review on the processing steps involved in defatting and extrusion was provided by Juliano (1985). While extrusion cooking has been reported to enhance the formation of phytic acid-protein complexes (Fuh and Chiang, 2001), it may also have a positive effect on amino acid digestibility. Sauer et al. (1990) reported increases of 2.5 and 4.5 percentage units for the fecal digestibilities of GE and CP, respectively, when diets for early weaned pigs were extruded, as compared to a pelleted diet. The ADG and feed conversion efficiency were also better ($P < 0.05$) with the extruded than with the pelleted diet. Freire et al. (1991) reported that extrusion increased the apparent ileal digestibility of nitrogen in a diet containing winter peas from 65.7 to 78.4 %.

Previous studies carried out with wheat shorts (Huang et al., 1999), canola meal (Fan et al., 1996), and wheat (Fan et al., 2001) have shown significant negative correlations between the apparent ileal digestibilities of CP and amino acids (in most instances) and the NDF content of these ingredients. The lower CP and amino acid digestibilities with increasing dietary fiber content may be attributed to an increase in the rate of passage of digesta (Stanogias and Pearce, 1985), adsorption of amino acids and peptides to fiber, increased losses of endogenous CP and amino acids, and a lower digestibility of CP and amino acids associated with fiber (Donangelo and Eggum, 1985). The NDF content in the samples of RB ranged from 2.1 to 34.3 % (Table 2-2). Simple regression equations were established between the apparent ileal digestibilities of CP and amino acids and the NDF content in the samples of RB (Table 2-7). For reasons of simplicity and practicality, only the regression equations for lysine, the sulphur-

containing amino acids, threonine, and tryptophan are presented. The regression equations for all amino acids are presented in the Appendix. With the exception of cystine ($P < 0.05$), there were no significant negative correlations. When RB 4, the atypical source of RB, was not included there were no significant correlations between the NDF content (25.5 to 34.3 %) and all the amino acids presented.

Linear relationships were also established between the dietary CF, as well as between the dietary ash and CP contents and the amino acid digestibility values (Tables 2-7 and 2-8). There were significant ($P < 0.05$) positive correlations with CF content (4.1 to 24.4 %) for lysine and tryptophan but not for the sulfur-containing amino acids and threonine (Table 2-7). Not including RB 4, there were significant positive correlations between the CF content (4.1 to 24.4%) and the digestibilities of the amino acids listed, except tryptophan. The significant positive correlations between apparent ileal digestibility of the amino acids presented and the CF content (excluding RB 4) are not surprising. Studies with young pigs (Li and Sauer, 1994) and growing pigs (Imbeah and Sauer, 1991) showed that the apparent ileal digestibilities of most amino acids increased with increasing dietary fat content.

There were no significant relationships between the ash content (4.8 to 21.0%) and the amino acid digestibilities (Table 2-8). Not including RB 4, there were positive relationships between the ash content (11.0 to 21.0%) and the digestibilities of the amino acids presented. Furthermore, with the exception of lysine and tryptophan (which approached significance, $P < 0.10$), there were significant ($P < 0.05$) negative correlations between the dietary CP content (13.6 to 21.0%) and the amino acid digestibility values

(Table 2-8). Not including RB 4, the negative correlations between the dietary CP content (15.6 to 21.0%) and the digestibilities were significant for all amino acids listed.

It is rather difficult to explain the positive effect of ash content and negative effect of CP content on amino acid digestibility values among the RB samples. Fan et al. (2001), in a study with pigs fed six sources of wheat, reported no differences ($P < 0.05$) between the CP content and apparent ileal digestibility values. However, in another study with six sources of canola meal, Fan et al. (1996) reported positive correlations ($P < 0.05$) between the CP content and apparent ileal digestibility values. Oddly, the apparent ileal digestibilities of the amino acids presented were negatively correlated with the CP content of RB, even though the NDF content in RB was not responsible for any significant variation. These results perhaps suggest the presence of considerable amounts of CP and amino acids associated with NDF in RB and which are of low digestibility.

At this point a comment should be made on the regression equations established in Tables 2-7 and 2-8. As a measure of the accuracy of a regression equation, the r^2 value represents the amount of variation that the variable X (NDF, CF, ash, or CP) in the RB sample contributes to the apparent ileal digestibility values (Y) for the same sample. Larger r^2 values would be obtained when the means of the digestibility values of CP and the individual amino acids were used to determine the regression equations. Values for r^2 based on means, rather than the smaller values based on individual observations as in these studies, are not correct and these values are often reported in the literature. Therefore, the data are not as closely fitted to the regression line as they would be when the means are used, although the regression equations are identical.

In summary, the present results indicate that some of the variation in amino acid digestibility values can be attributed to the dietary CF, ash, and CP content. These studies show that there is considerable variation in CP and amino acid digestibility values in RB, and that it is rather difficult to specify an overriding factor responsible for the variation.

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Table 2-1. Formulation (%) of the experimental diets[a]

Ingredient	Diets					
	A	B	C	D	E	F
Corn	53.80	53.80	53.80	53.80	53.80	71.03
Soybean meal	19.90	19.90	19.90	19.90	19.90	26.30
Rice bran	23.10[b]	23.10[c]	23.10[d]	23.10[e]	23.10[f]	-
Limestone	1.80	1.80	1.80	1.80	1.80	1.07
Dicalcium phosphate	0.60	0.60	0.60	0.60	0.60	0.75
Choline chloride[g]	0.05	0.05	0.05	0.05	0.05	0.05
Trace-mineralizedsalt[h]	0.30	0.30	0.30	0.30	0.30	0.30
Mineral premix[i]	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin premix[j]	0.10	0.10	0.10	0.10	0.10	0.10
Chromic oxide[k]	0.30	0.30	0.30	0.30	0.30	0.30

[a]As fed basis
[b]Rice bran 1
[c]Rice bran 2
[d]Rice bran 3
[e]Rice bran 4
[f]Rice bran 5
[g]Contained 521,000 mg of choline/kg of choline chloride. Supplied by Champion Feed Services, Westlock, AB.
[h]Composition (%): NaCl, 96.5; ZnO, 0.40; $FeCO_3$, 0.16; MnO, 0.12; CuO, 0.033; $Ca(IO_3)_2$, 0.007; CaO, 0.004. Supplied by Windsor Salt, Toronto, ON.
[i]Contained the following (per kilogram of premix): Fe, 150,000 mg; Zn, 150,000 mg; Cu, 25,000 mg; Mn 40,000 mg; Co, 500 mg; I, 500 mg; Se, 500 mg. Supplied by Champion Feed Services, Westlock, AB.
[j]Contained the following (per kilogram of premix): vitamin A, 10,000 KIU; vitamin D3, 1,100 KIU; vitamin E, 30,000 IU; vitamin K, 3,000 mg; vitamin B12, 20,000 µg; thiamine, 1,000 mg; riboflavin, 4,000 mg; niacin, 30,000 mg; pantothenic acid, 15,000 mg; pyridoxine, 2,000 mg. Supplied by Champion Feed Services, Westlock, AB.
[k]Fisher Scientific, Fair Lawn, NJ.

Table 2-2. Chemical and amino acid composition (%) of the ingredients in the experimental diets[a]

Item	Ingredients						
	Corn	Soybean meal	Rice bran 1	Rice bran 2	Rice bran 3	Rice bran 4	Rice bran 5
Dry matter	83.8	88.1	93.1	90.6	90.1	86.5	91.6
Energy, kcal/kg	4,544.7	4,868.2	4,906.5	4,476.3	5,677.4	4,593.2	5,057.4
Crude fat	4.2	1.7	23.4	4.1	24.4	5.4	20.7
Neutral detergent fiber	9.9	11.2	26.4	26.0	34.3	2.1	25.5
Ash	2.2	7.4	21.0	12.7	11.0	4.8	16.3
Crude protein	10.2	52.87	15.6	21.0	15.8	13.6	18.1
Amino acids							
Indispensable							
Arginine	0.47	3.82	1.32	1.73	1.29	0.99	1.45
Histidine	0.27	1.44	0.44	0.61	0.44	0.34	0.50
Isoleucine	0.33	2.19	0.51	0.68	0.56	0.44	0.59
Leucine	1.14	3.95	1.05	1.38	1.14	0.94	1.22
Lysine	0.30	3.13	0.69	0.88	0.75	0.45	0.76
Methionine	0.22	0.70	0.30	0.40	0.33	0.31	0.34
Phenylalanine	0.48	2.60	0.65	0.86	0.71	0.60	0.76
Threonine	0.36	2.11	0.56	0.75	0.62	0.46	0.65
Valine	0.45	2.27	0.80	1.04	0.87	0.64	0.89
Tryptophan	0.08	0.71	0.20	0.28	0.20	0.17	0.22
Dispensable							
Alanine	0.70	2.24	0.91	1.22	0.98	0.72	1.03
Aspartic acid	0.69	6.00	1.25	1.75	1.33	1.08	1.46
Cystine	0.20	0.75	0.27	0.40	0.30	0.28	0.32
Glutamic acid	1.82	9.45	1.97	2.81	2.01	2.08	2.41
Glycine	0.38	2.17	0.82	1.09	0.88	0.58	0.94
Serine	0.49	2.77	0.71	0.93	0.74	0.62	0.82
Proline	0.89	2.71	0.69	0.89	0.74	0.60	0.82

[a]Dry matter basis

Table 2-3. The linear relationships between the contents of crude protein and amino acids in different sources of rice bran

Item	Regression equations	r^2	P^a	P^b
Amino acids				
Indispensable				
Arginine	$Y^c = 0.0825 - 0.1958X^d$	0.96	0.3284	0.004
Histidine	$Y = 0.0308 - 0.1088X$	0.98	0.0731	0.001
Isoleucine	$Y = 0.0271 + 0.0347X$	0.96	0.5678	0.003
Leucine	$Y = 0.0502 + 0.1611X$	0.97	0.1765	0.003
Lysine	$Y = 0.0449 + 0.134X$	0.79	0.6001	0.045
Methionine	$Y = 0.0114 + 0.1025X$	0.85	0.1179	0.026
Phenylalanine	$Y = 0.0298 + 0.1259X$	0.97	0.0999	0.003
Threonine	$Y = 0.0324 - 0.0104X$	0.93	0.9128	0.008
Valine	$Y = 0.0439 + 0.0085X$	0.91	0.9533	0.011
Tryptophan	$Y = 0.0116 - 0.0093X$	0.96	0.7136	0.003
Dispensable				
Alanine	$Y = 0.0555 - 0.0808X$	0.94	0.6101	0.007
Aspartic acid	$Y = 0.0773 - 0.0921X$	0.99	0.3655	0.001
Cystine	$Y = 0.0150 + 0.0238X$	0.89	0.6733	0.016
Glutamic acid	$Y = 0.1019 + 0.2704X$	0.84	0.5781	0.028
Glycine	$Y = 0.0542 - 0.1548X$	0.89	0.4765	0.017
Serine	$Y = 0.0371 + 0.0463X$	0.99	0.3085	0.001
Proline	$Y = 0.0332 + 0.0989X$	0.95	0.2772	0.005

[a] The probabilities of significance for the intercepts of the regression equations.
[b] The probabilities of significance for the slopes of the regression equations.
[c] Y represents the content of a particular amino acid in the rice bran sample (%).
[d] X represents content (%) of CP in the rice bran sample.

Table 2-4. Chemical and amino acid composition (%) of the experimental diets[a]

	Diets					
	A	B	C	D	E	F
Corn, %	53.80	53.80	53.80	53.80	53.80	71.03
Soybean meal, %	19.90	19.90	19.90	19.90	19.90	26.30
Rice bran, %	23.10	23.10	23.10	23.10	23.10	-
Item						
Dry matter	89.3	88.8	88.7	89.3	89.5	87.2
Energy, kcal/kg	4,530.6	4,403.8	4,718.1	4,343.6	4,563.4	4,464.2
Crude fat	7.5	3.3	7.9	3.3	7.7	3.2
Neutral detergent fiber	13.0	13.0	14.8	7.0	11.7	8.2
Ash	10.4	9.2	8.4	7.1	9.6	6.5
Crude protein	18.0	20.2	18.6	19.3	20.5	20.5
Amino acids						
Indispensable						
Arginine	1.26	1.43	1.28	1.28	1.32	1.18
Histidine	0.51	0.57	0.52	0.51	0.53	0.51
Isoleucine	0.72	0.82	0.78	0.77	0.78	0.78
Leucine	1.59	1.73	1.67	1.69	1.68	1.77
Lysine	0.90	1.00	0.94	0.90	0.93	0.90
Methionine	0.32	0.36	0.34	0.34	0.34	0.32
Phenylalanine	0.90	0.99	0.94	0.96	0.94	0.95
Threonine	0.71	0.78	0.74	0.73	0.73	0.72
Valine	0.88	1.00	0.94	0.90	0.95	0.90
Tryptophan	0.23	0.25	0.23	0.22	0.23	0.22
Dispensable						
Alanine	1.01	1.11	1.05	1.03	1.06	1.03
Aspartic acid	1.75	1.99	1.84	1.87	1.84	1.83
Cystine	0.31	0.35	0.32	0.33	0.33	0.31
Glutamic acid	3.20	3.53	3.32	3.48	3.34	3.48
Glycine	0.80	0.90	0.83	0.80	0.84	0.76
Serine	0.92	1.00	0.94	0.97	0.94	0.93
Proline	1.13	1.17	1.19	1.17	1.23	1.25

[a]Dry matter basis

Table 2-5. Apparent ileal digestibilities (%) of dry matter, crude protein and amino acids, and energy digestibility (%) in the experimental diets

	Diets						
	A	B	C	D	E	F	
Corn, %	53.80	53.80	53.80	53.80	53.80	71.03	
Soybean meal, %	19.90	19.90	19.90	19.90	19.90	26.30	
Rice bran, %	23.10	23.10	23.10	23.10	23.10	-	
Item							SEM[a]
Dry matter	59.1^{cd}	56.4^{d}	58.2^{cd}	68.7^{b}	61.1^{c}	61.2^{c}	0.93
Energy	81.1^{d}	82.7^{d}	81.1^{d}	88.2^{b}	82.5^{d}	85.7^{c}	0.39
Crude protein	71.0^{b}	64.8^{c}	68.5^{b}	72.0^{b}	71.9^{b}	71.1^{b}	0.85
Amino acids							
Indispensable							
Arginine	87.8^{b}	84.6^{d}	86.5^{bc}	87.6^{b}	87.2^{bc}	86.0^{c}	0.40
Histidine	82.4^{b}	78.0^{c}	81.1^{b}	82.1^{b}	81.2^{b}	80.0^{b}	0.60
Isoleucine	80.2^{b}	75.8^{c}	79.1^{b}	80.5^{b}	79.8^{b}	79.1^{b}	0.78
Leucine	82.9^{b}	78.2^{c}	81.4^{b}	83.4^{b}	81.8^{b}	82.3^{b}	0.60
Lysine	81.6^{b}	76.9^{c}	79.6^{bc}	80.0^{bc}	79.6^{bc}	78.8^{bc}	0.73
Methionine	84.1^{b}	79.5^{c}	81.7^{b}	83.3^{b}	83.4^{b}	83.8^{b}	0.62
Phenylalanine	80.0^{b}	75.3^{c}	78.6^{b}	81.0^{b}	79.5^{b}	79.2^{bc}	0.62
Threonine	73.5^{b}	68.0^{c}	71.7^{b}	73.5^{b}	72.2^{b}	69.8^{bc}	0.90
Valine	78.3^{b}	73.9^{c}	77.1^{b}	78.5^{b}	78.0^{b}	76.4^{b}	0.82
Tryptophan	76.5^{b}	70.1^{cd}	73.6^{bc}	73.8^{bc}	74.4^{bc}	72.3^{cd}	0.91
Dispensable							
Alanine	79.6^{b}	74.4^{c}	77.2^{b}	79.0^{b}	78.0^{b}	76.9^{b}	0.75
Aspartic acid	78.3^{b}	72.8^{c}	76.6^{b}	79.2^{b}	77.3^{b}	76.7^{b}	0.66
Cystine	75.1^{bc}	67.5^{d}	72.6^{bc}	75.5^{b}	74.6^{bc}	71.6^{c}	0.89
Glutamic acid	85.3^{b}	80.2^{c}	83.4^{b}	85.2^{b}	84.2^{b}	83.7^{b}	0.62
Glycine	71.5^{b}	63.6^{c}	67.4^{bc}	70.8^{b}	70.0^{b}	66.8^{bc}	1.10
Serine	79.0^{bc}	73.8^{d}	77.1^{bc}	79.4^{b}	77.7^{bc}	76.8^{c}	0.61
Proline	80.0^{b}	75.0^{c}	78.3^{b}	80.6^{b}	80.7^{b}	79.1^{b}	0.79

[a]Standard error of the mean

[b,c,d]Means in the same row with different superscript letters differ ($P < 0.05$)

Table 2-6. Apparent ileal digestibilities (%) of dry matter, crude protein and amino acids, and fecal energy digestibility (%) in the samples of rice bran

Item	Ingredients					
	Rice bran 1	Rice bran 2	Rice bran 3	Rice bran 4	Rice bran 5	SEM[a]
Dry matter	49.7^{cd}	35.8^{e}	43.0^{cd}	85.9^{b}	55.0^{c}	4.23
Energy	60.5^{c}	64.1^{c}	62.6^{c}	86.9^{b}	65.8^{c}	1.42
Crude protein	61.3^{b}	38.3^{c}	48.3^{c}	65.3^{b}	67.3^{b}	3.88
Amino acids						
Indispensable						
Arginine	85.1^{b}	75.7^{c}	79.7^{b}	83.1^{b}	83.3^{b}	1.29
Histidine	81.7^{b}	66.0^{c}	75.8^{b}	79.8^{b}	76.7^{b}	2.20
Isoleucine	73.2^{b}	53.9^{c}	67.7^{b}	73.4^{b}	72.0^{b}	3.65
Leucine	72.0^{b}	50.9^{c}	63.7^{b}	73.5^{b}	67.2^{b}	3.11
Lysine	82.2^{b}	62.6^{c}	72.7^{bc}	70.5^{bc}	73.1^{bc}	3.47
Methionine	76.0^{b}	61.4^{c}	66.9^{bc}	72.2^{b}	74.0^{b}	2.32
Phenylalanine	71.9^{bc}	52.5^{d}	65.2^{c}	76.8^{b}	70.6^{bc}	2.81
Threonine	82.8^{b}	58.4^{c}	73.9^{b}	86.1^{b}	75.9^{b}	3.72
Valine	76.2^{b}	60.2^{c}	71.1^{b}	76.9^{b}	75.3^{b}	3.04
Tryptophan	82.6^{b}	58.8^{c}	69.7^{bc}	70.0^{bc}	73.3^{b}	3.27
Dispensable						
Alanine	80.2^{b}	61.1^{c}	69.9^{bc}	77.0^{b}	73.6^{b}	2.96
Aspartic acid	73.9^{bc}	51.6^{d}	65.2^{c}	79.4^{b}	69.8^{bc}	3.05
Cystine	79.1^{b}	51.6^{d}	62.5^{c}	81.0^{b}	76.3^{b}	3.38
Glutamic acid	78.5^{b}	55.6^{d}	66.3^{c}	78.0^{b}	74.0^{bc}	2.93
Glycine	78.5^{b}	52.3^{d}	63.1^{cd}	78.4^{b}	72.5^{bc}	3.78
Serine	77.6^{bc}	56.5^{d}	68.2^{c}	80.1^{b}	71.9^{bc}	2.62
Proline	71.0^{b}	45.2^{c}	59.9^{bc}	72.8^{b}	76.0^{b}	4.92

[a]Standard error of the mean

[b,c,d,e]Means in the same row with different superscript letters differ ($P < .05$)

Table 2-7. The linear relationships between apparent ileal digestibilities of selected amino acids and dietary neutral detergent fiber or crude fat content

Amino Acids	Linear relationship with neutral detergent fiber	r^2	P^c	P^d
Lysine	$Y^a = 70.35 + 0.08X^b$	0.0059	< 0.0001	0.6861
Methionine	$Y = 73.07 - 0.13X$	0.0304	< 0.0001	0.3565
Cystine	$Y = 82.53 - 0.54X$	0.1378	< 0.0001	0.0434
Threonine	$Y = 85.64 - 0.45X$	0.1137	< 0.0001	0.0684
Tryptophan	$Y = 70.24 + 0.03X$	0.0004	< 0.0001	0.9114
	Linear relationship with neutral detergent fiber (excluding rice bran 4)			
Lysine	$Y = 70.19 + 0.09X$	0.0008	0.0013	0.8978
Methionine	$Y = 81.21 - 0.41X$	0.0322	0.0001	0.4012
Cystine	$Y = 88.35 - 0.75X$	0.0277	0.0032	0.4368
Threonine	$Y = 65.66 + 0.25X$	0.0041	0.0109	0.7654
Tryptophan	$Y = 74.90 - 0.14X$	0.0012	0.0041	0.8707
	Linear relationship with crude fat			
Lysine	$Y = 63.83 + 0.54X$	0.1725	< 0.0001	0.0225
Methionine	$Y = 65.59 + 0.29X$	0.1036	< 0.0001	0.0829
Cystine	$Y = 64.75 + 0.34X$	0.0371	< 0.0001	0.3076
Threonine	$Y = 70.20 + 0.33X$	0.0431	< 0.0001	0.2712
Tryptophan	$Y = 61.44 + 0.60X$	0.1548	< 0.0001	0.0315
	Linear relationship with crude fat (excluding rice bran 4)			
Lysine	$Y = 59.66 + 0.72X$	0.2661	< 0.0001	0.0099
Methionine	$Y = 60.02 + 0.53X$	0.2710	< 0.0001	0.0091
Cystine	$Y = 48.93 + 1.02X$	0.2667	< 0.0001	0.0098
Threonine	$Y = 54.69 + 0.99X$	0.3342	< 0.0001	0.0031
Tryptophan	$Y = 55.65 + 0.85X$	0.2490	< 0.0001	0.0138

[a] Y represents digestibility coefficient (%). [b] X represents content (%) of neutral detergent fiber or crude fat in the rice bran.
[c] The probabilities of significance for the intercepts of the regression equations.
[d] The probabilities of significance for the slopes of the regression equations.

Table 2-8. The linear relationships between apparent ileal digestibilities of selected amino acids and dietary ash or crude protein content

Amino Acids	Linear relationship with ash	r^2	P^c	P^d
Lysine	$Y^a = 63.35 + 0.68X^b$	0.0995	< 0.0001	0.0895
Methionine	Y = 65.43 + 0.36X	0.0573	< 0.0001	0.2025
Cystine	Y = 67.93 + 0.17X	0.0032	< 0.0001	0.7680
Threonine	Y = 77.13 - 0.13X	0.0024	< 0.0001	0.7960
Tryptophan	Y = 60.55 + 0.78X	0.0958	< 0.0001	0.0961
	Linear relationship with ash (excluding rice bran 4)			
Lysine	Y = 51.59 + 1.38X	0.2154	< 0.0001	0.0223
Methionine	Y = 50.34 + 1.26X	0.3379	< 0.0001	0.0029
Cystine	Y = 31.63 + 2.35X	0.3085	0.0134	0.0048
Threonine	Y = 48.75 + 1.58X	0.1818	0.0003	0.0377
Tryptophan	Y = 43.70 + 1.80X	0.2416	0.0005	0.0147
	Linear relationship with crude protein			
Lysine	Y = 96.80 – 1.46X	0.1022	< 0.0001	0.0850
Methionine	Y = 92.29 – 1.32X	0.1728	< 0.0001	0.0223
Cystine	Y = 126.72 - 3.36X	0.2885	< 0.0001	0.0022
Threonine	Y = 134.38 – 3.50X	0.3819	< 0.0001	0.0003
Tryptophan	Y = 101.29 – 1.81X	0.1121	< 0.0001	0.0705
	Linear relationship with crude protein (excluding rice bran 4)			
Lysine	Y = 121.87 – 2.79X	0.2883	< 0.0001	0.0068
Methionine	Y = 100.64 – 1.76X	0.2159	< 0.0001	0.0221
Cystine	Y = 126.57 - 3.36X	0.2072	< 0.0001	0.0254
Threonine	Y = 136.72 – 3.63X	0.3169	< 0.0001	0.0042
Tryptophan	Y = 127.58 – 3.20X	0.2524	< 0.0001	0.0124

[a] Y represents digestibility coefficient (%). [b] X represents content (%) of ash or crude protein in the rice bran.
[c] The probabilities of significance for the intercepts of the regression equations.
[d] The probabilities of significance for the slopes of the regression equations.

CHAPTER 3

APPARENT ILEAL DIGESTIBILITIES OF NEUTRAL-DETERGENT FIBER, CRUDE PROTEIN AND AMINO ACIDS ASSOCIATED WITH NEUTRAL-DETERGENT FIBER IN RICE BRAN FED TO GROWING PIGS

A. Introduction

Rice bran (**RB**) is the by-product of the rice-milling industry when brown rice is refined into white rice for human consumption. The four layers of the caryopsis, which are the pericarp, seed coat, nucellus and the aleurone, in addition to most of the embryo, are designated RB.

There is considerable variation in the chemical composition and digestibility of RB, and Warren and Farrell (1990a) surmised that this may be due to varying proportions of hulls in RB, which have little nutritional value. It was established that differences in neutral detergent fiber (**NDF**) levels are a cause of variation in apparent ileal amino acid digestibilities between different samples of wheat shorts (Huang et al., 1999). Schulze et al. (1994) reported that the passage of undigested nitrogen increases as the NDF content in the diet increases. It was reported that wheat bran contains approximately 7 g of nitrogen / kg of which over 4 g is associated with NDF (Lenis et al., 1996). Nitrogen and amino acids associated with the NDF fraction are likely to be of low digestibility because the digestive enzymes have limited access to the cell wall components and contents (Bjergegaard et al., 1991). In a previous study, it was reported that a considerable proportion of CP and amino acids in wheat shorts are associated with the NDF fraction (Huang et al., 2001).

The objectives of this study were to determine 1) the contents of CP and amino acids associated with NDF in five different sources of RB, and 2) the apparent ileal CP and amino acid digestibility of CP and amino acids associated with NDF. In addition, a relationship was established between the digestibilities of CP and amino acids and NDF-associated CP.

B. Materials and Methods

Animals and Diets

Six barrows, average initial BW of 36.5 kg, were obtained from the University of Alberta Swine Research and Technology Centre. The animals were individually confined in stainless steel metabolic crates in a temperature-controlled facility (20 to 22°C). Following a 7-d adaptation period to the metabolic crates, the barrows were fitted with a simple T-cannula at the distal ileum according to procedures adapted from Sauer et al. (1983). The cannulas were modified according to de Lange et al. (1989). A detailed description of pre- and post-operative care was previously presented by Li et al. (1994). A 12-d recuperation period followed, and during this time, the barrows were fed an 18 % CP diet (Table 2-1, Diet F).

After recuperation, the barrows were fed one of six experimental diets (Table 1) according to a 6 × 6 Latin square design. They were given two meals of equal amounts each day, at 0800 and 2000, with the daily dietary allowance equivalent to 4 % (wt/wt) of the individual BW determined at the beginning of each experimental period. Water was available at all times from a low-pressure drinking nipple.

Six diets were formulated (Table 2-1) to contain 18 % CP (% N × 6.25, as-fed basis). Diets A, B, C, D and E contained 53.8 % corn, 19.9 % soybean meal (**SBM**) and

23.1 % RB. Five different sources of RB were obtained. Rice bran 1: Canadian source of RB; RB 2: Riceland Foods Inc., Stuttgart, AR, 72160; RB 3: Agribrands International, Italy; RB 4: North Luzon Region, Phillipines; and RB 5: Agribrands/Tradico, (Greenville Feed Facility) Greenville, MS., 38701. Diet F contained 71.03 % corn and 26.3 % SBM. Vitamins and minerals were supplemented to meet or exceed NRC (1998) standards, and chromic oxide was included in the diets at .3 % as the digestibility marker. The diets were fed in a mash form.

The duration of each experimental period was 11 d. The collection of feces was initiated at 0800 on d 8 of each experimental period and continued for 48 h. Ileal digesta were collected for a total of 24 h; from 0800 to 2000 on d 9 and from 2000 on d 10 to 0800 on d 11. The procedure for digesta collection was adapted from Li et al. (1994). Feces and digesta were frozen at -28°C immediately after collection, and the samples were pooled within barrows and periods. The barrows used in this experiment were cared for in accordance with the guidelines established by CCAC (1993) and approved by the Faculty of Agriculture, Forestry and Home Economics Animal Care Committee of the University of Alberta.

Chemical Analyses

Prior to analysis, samples of ingredients, diets, oven-dried feces and freeze-dried digesta were ground through a 1-mm mesh screen using a Wiley mill (Arthur H. Thomas Co., Philadelphia, PA).

Neutral detergent fiber was isolated using procedures adapted from Goering and Van Soest (1970), and a description of techniques is given by Huang et al. (2001). Briefly, about 1 g of the diets, dietary ingredients or ileal digesta was weighed into a 600

ml beaker designed for use with a refluxing apparatus. Neutral detergent fiber solution (100 ml) and 100 µL of heat-stable α-amylase (EC 3.2.1.1; Sigma Chemical Company, St. Louis, MO) were added to the beaker, and this mixture was then kept at room temperature overnight. The contents were heated to boiling on a refluxing apparatus for 60 min, after which the solution was filtered into a Buchner funnel lined with filter paper connected to an aspirator. The filtered material was washed several times with hot water to remove any NDF solution, followed by several washes with acetone, then removed from the paper and dried in an oven at 110°C overnight. Approximately 1 g of NDF per sample was isolated in total. Due to inaccuracies associated with using filter paper, the NDF content in the samples had to be determined using the filter-bag technique (Ankom Co., Fairport, NY). Approximately .5 g of each sample was measured into a filter bag, which was then heat-sealed. Each sample was submerged overnight in 100 ml NDF solution with 100 µL of α-amylase. The bags were placed on a bag suspender in 100 ml of NDF solution per sample at 97 to 99°C. After agitating for 70 min, the bags were removed and immediately rinsed in water (80-99°C) twice, followed by several more rinses with the addition of 100 µl of α-amylase per sample. This procedure was repeated until no starch remained as determined with the iodine test. The bags were then soaked for 2 to 3 min. in acetone, air-dried, and placed in an oven at 110°C overnight. Chromic oxide was measured according to Fenton and Fenton (1979).

Amino acid and CP analyses were carried out at Degussa AG, Hanau, F.R.G. Prior to analyses, samples were ground through a 0.5-mm mesh screen. The nitrogen content of the NDF samples was determined according to AOAC 2000 procedures using a Leco Nitrogen Analyzer (Leco Corporation, St. Joseph, MI). For the analyses of amino

acids, except for tryptophan and tyrosine which were not determined, the following procedure was used. A sample containing approximately 10 mg nitrogen (the total quantity of sample may vary from 100 to 800 mg) was placed into a 50 ml flask. Then, 5 ml of cold performic acid was added and the solution was stirred with a magnetic rod for 15 min while in an ice bath. The flask was then closed and allowed to react for 16 h at 0°C to yield methionine sulfone and cysteic acid. Following, excess performic acid was destroyed by adding about 0.84 g sodium disulphite, while stirring for 30 min and exhausting any sulphur dioxide into a fume hood. Thereafter, hydrolysis was initiated by adding 25 ml hydrochloric acid-phenol reagent into the flask and placing it in an oven at 110°C for 24 h.

After hydrolysis, the flasks were cooled and 15 ml norleucine standard solution was pipetted into each flask. After mixing, the hydrolysate is transferred to a 250 ml beaker, to which 125 ml citrate buffer and 19 ml sodium hydroxide solution are added. Sodium hydroxide solution (1 mol/L) was used to adjust the pH to 2.2. A portion of the resulting solution was transferred to a 50 ml flask, and frozen until analysis. This solution was then applied to the column of the amino acid analyzer. The amino acid analyzer was a Biochrom 20 with a two-channel A/D converter card. Amino acid chromatograms are simultaneously detected at 570 and 440 nm. The EZ Chrom™ integration software (Scientific Software Inc., USA) is used for integration of the chromatograms.

For the oxidation mixture, an 88 % phenolic-formic acid solution was prepared by mixing 889 g formic acid with 111 g distilled or deionized water and approximately 5 g phenol. The final oxidation mixture was prepared by mixing 10 ml 30 % hydrogen

peroxide with 90 ml of the phenolic-formic acid solution in a 100 ml Erlenmeyer flask. Thereafter, the flask was sealed and allowed to stand for 1 h at 20 to 30°C to ensure the formation of performic acid. The hydrochloric acid-phenol hydrolysis reagent (6 M HCl) was formulated by dissolving 5 g phenol in 2,500 ml water in a 5,000 ml bottle. Then, 2,500 ml of 37 % hydrochloric acid was added and mixed. The norleucine standard was prepared by dissolving 2 g norleucine in 500 ml of 1 mol/L hydrochloric acid, and topped up with water in a 5,000 ml volumetric flask. The citrate buffer requires 98 g sodium citrate and 5 g phenol to be dissolved in 4,000 ml water in a 5,000ml beaker. Then, 25 ml thiodiglycol and 80 ml 37 % hydrochloric acid were added, and this was diluted with 800 ml water. The pH was adjusted to 2.2 with hydrochloric acid, and the mixture was transferred to a 5,000 ml volumetric flask, which was topped up with water. The sodium hydroxide solution (7.5 mol/L) was prepared by weighing 937 g of 32 % sodium hydroxide solution into a 1,000 ml volumetric flask while stirring, and then topped up with water.

Analyses of ingredients and diets were performed in triplicate, and digesta and feces in duplicate.

Calculations and Statistical Analysis

The apparent ileal digestibilities of NDF in the experimental diets were determined using the indicator technique, which is represented by Equation 1.

$$D_D = 100\% - [(I_D \times A_F) / (I_F \times A_D)] \times 100\% \qquad \text{(Equation 1)}$$

Where D_D: apparent digestibility value of NDF in the assay diet (%); I_D: indicator concentration in the assay diet (g kg^{-1}); A_F: concentration of NDF in ileal digesta (g kg^{-1});

I_F: indicator concentration in ileal digesta (g kg^{-1}); A_D: concentration of NDF in the assay diet (g kg^{-1}).

The ileal digestibilities of CP and amino acids associated with NDF were calculated using Equation 2.

$$D_N = ((N_D - N_I) / N_D) \times 100\ \% \qquad \text{(Equation 2)}$$

Where D_N: true ileal digestibility of NDF-associated CP or amino acids in the assay diet (%); N_D: intake of NDF-associated CP or amino acids in the assay diet (g kg^{-1}); N_I: recovery of NDF-associated CP or amino acids in ileal digesta (g kg^{-1}).

Statistical analysis of the digestibility values of the amino acids and CP associated with NDF in the experimental diets was performed as a 6 × 6 Latin square using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Means of the dietary treatments, experimental periods and pigs were compared using the Student-Newman Keul's multiple range test. Relationships between the NDF-associated CP content and apparent ileal digestibility values of CP and amino acids in RB were determined using the simple linear regression procedure of SAS.

C. Results and Discussion

The amino acid contents of corn, SBM and the five RB samples were previously presented in Chapter II. The contents of CP and amino acids associated with NDF and their contribution to total CP and amino acids in the dietary ingredients are presented in Table 3-1. The levels of CP and amino acids associated with NDF from RB were lowest in RB 3 and highest in RB 2. The content of NDF-associated CP ranged from 7.87% in RB 3 to 16.58% in RB 2. The levels of lysine, methionine, and threonine associated with NDF in the samples of RB ranged from 0.18 to 0.60 %, from 0.20 to 0.38 %, and from

0.28 to 0.61 %, respectively. The aforementioned amino acids are often referred to, as these are usually first-, second-, or third-limiting in diets for pigs. The NDF contents were 26.4, 26.0, 34.3, 2.1, and 25.5% in RB 1, RB 2, RB 3, RB 4, and RB 5, respectively. Not including RB 4, the atypical source of RB (Chapter II), the contribution of CP associated with NDF in RB to the total CP in RB ranged from 16.00 % in RB 1 to 20.47 % in RB 2; the contributions of lysine, methionine, and threonine ranged from 11.88 to 17.71 %, from 20.78 to 24.68 %, and from 17.47 to 21.12 %, respectively. Of the indispensable amino acids, the contribution was usually highest for methionine and lowest for arginine. In comparison, Huang et al. (2001) reported levels of CP, lysine, methionine, and threonine associated with NDF in wheat bran to be 5.37, 0.25, 0.12, and 0.22 %, respectively. The contributions of CP, lysine, methionine, and threonine associated with NDF to the total levels of these in wheat bran were 14.0, 19.1, 19.3, and 18.4 %, respectively. It is evident that the content of NDF-associated CP, as well as its contribution to total CP content, is larger for RB than for wheat bran. The contents and contributions of NDF-associated lysine, methionine, and threonine were similar between RB and wheat bran.

The contents of CP and amino acid associated with NDF and their contribution to the total CP and amino acid content in the RB-containing diets are presented in Table 3-2. Not including diet D, which included RB 4, the content of CP associated with NDF ranged from 7.31 to 11.31 %. The contents of lysine, methionine, and threonine ranged from 0.23 to 0.38 %, from 0.14 to 0.22 %, and from 0.29 to 0.43 %, respectively. The contribution of NDF-associated CP to total CP content in the RB-containing diets ranged

from 5.36 to 7.32 %. The contributions of lysine, methionine, and threonine ranged from 3.40 to 4.96 %, from 5.52 to 7.98 %, and from 5.46 to 7.20 %, respectively.

The contributions of CP and amino acids associated with NDF to total CP and amino acids in ileal digesta of pigs fed the experimental diets are presented in Table 3-3. Not including diet D, the contributions of CP were nearly the same and ranged from 11.38 to 12.77 %; the contributions of lysine, methionine, and threonine ranged from 11.58 to 12.51 %, from 13.53 to 18.34 %, and from 10.18 to 11.91 %, respectively.

The apparent ileal digestibility values for NDF and NDF-associated CP and amino acids are presented in Table 3-4. The ileal digestibility values of NDF in the RB-containing diets were very low, in two cases negative, and ranged from -8.1 to 16.0 %. Not including diet D, the digestibility values of NDF ranged from -3.6 to 16.0 %. However, when the ileal digestibility values of NDF from the RB-containing diets were compared to 0 digestibility, only diet A differed ($P < 0.05$). Huang et al. (2001) reported ileal digestibility values of NDF of 11.8 to 13.9 % in diets containing varying levels of wheat bran, shorts , and flour. Schulze et al. (1994) reported values of 16.2 to 18.0 % for the apparent ileal digestibility of purified NDF isolated from wheat bran. The differences between the ileal digestibility values of NDF reported in the aforementioned studies likely result from differences in the composition of NDF, which consists mainly of cellulose, hemicellulose, and lignin. Buraczewska et al. (1988) reported a disappearance of 10 to 32 % of NDF in the small intestine of the pig depending on the source of NDF. They postulated that the hemicellulose fraction of NDF is most prone to bacterial fermentation in the small intestine. It should be mentioned that the presence of significant bacterial fermentation in the small intestine (as opposed to the large intestine)

has often been overlooked as was discussed by Wünsche et al. (1991), Dugan et al. (1994), and Huang et al. (2001). Keys et al. (1970) also reported that hemicellulose is more susceptible to bacterial fermentation than cellulose in the pig. Sandberg et al. (1981) carried out a study with ileostomized humans on the effect of fiber on nutrient absorption in the small intestine. After consumption of wheat bran, between 79 to 100 % of arabinose and between 69 to 106 % of xylose (components of hemicellulose) were recovered. The recovery of cellulose ranged from 74 to 100 %. Neutral detergent fiber from RB may contain relatively more cellulose and lignin than hemicellulose compared to other sources of NDF, and therefore its lower digestibility. Warren and Farrell (1990a) reported hemicellulose and lignin contents in RB ranging from 10.6 to 15.6 % and from 2.9 to 5.3 %, respectively. Furthermore, Dung and Uden (2002) reported that the degradation of NDF in pigs by using an *in vitro* method was lower in RB than in other feedstuffs, which they attributed to its high contents of lignin and silica, as silica depresses the digestibility of organic matter (Goering and Van Soest, 1970).

The apparent ileal digestibility values for NDF-associated CP and amino acids in the RB-containing diets varied considerably (Table 3-4). Not including diet D, the CP digestibilities ranged from 39.2 to 50.7 %; the digestibilities of lysine, methionine, and threonine ranged from 31.3 to 45.0 %, from 54.5 to 65.5 %, and from 44.4 to 54.5 %, respectively. Of the indispensable amino acids, the digestibility value of lysine was the lowest. Huang et al. (2001) reported apparent ileal digestibilities of CP associated with NDF from wheat fractions ranging from 66.3 to 72.8 %; the digestibilities of lysine, methionine, and threonine ranged from 56.3 to 64.6 %, from 44.0 to 51.8 %, and from 43.9 to 51.8 %, respectively. It should be pointed out that these digestibility values

represent true, rather than apparent, values. The apparent values will be lower because dietary fiber per se may affect the recovery of endogenous amino acids in ileal digesta and decrease amino acid absorption from dietary protein. Schulze et al. (1995) reported that the inclusion of 200 g NDF / kg diet increased the flow of endogenous nitrogen at the distal ileum and that the total increase in ileal nitrogen is greater when NDF is part of a dietary ingredient than when given in a purified form. As was pointed out previously, the level and source of fiber are two important factors affecting the recoveries of endogenous amino acids in ileal digesta (Sauer and Ozimek, 1986). The NDF fraction is made up of a heterogeneous mixture of structural (cellulose, hemicellulose, and pectins) and non-structural polysaccharides and lignin (Goering and Van Soest, 1970). The NDF fraction can also be partitioned into water-insoluble and water-soluble polysaccharides; the proportions dependent on the source of NDF. As was reviewed by Huang et al. (2001), the dietary inclusion of water-insoluble fiber sources will increase the recovery of endogenous amino acids in ileal digesta in pigs. However, the increases are not always significant. As was discussed by Li et al. (1994), the increases may become quantitatively important only when a certain level of inclusion (threshold level) is exceeded. In studies with rats, it was reported that the dietary inclusion of water-insoluble fiber increased the sloughing of intestinal mucosal cells and mucus production (Schneeman et al., 1982). Further, as was also reviewed by Huang et al. (2001), the dietary inclusion of water-soluble fiber, due to its gelling and viscosity properties, will decrease digestion and absorption of amino acids by reducing the mixing of intestinal contents, blocking enzyme-substrate interaction, and by forming an unstirred water layer, thereby creating a physical barrier to absorption. Finally, fiber may adsorb amino acids

and peptides, withholding these from absorption, the extent which depends on the degree of lignification (Bergner et al., 1975; Mitaru et al., 1984).

The linear relationships between the apparent ileal digestibilities of CP and amino acids and NDF-associated CP in the sources of RB are presented in Table 3-5. For the indispensable amino acids, with the exception of methionine, there were significant ($P < 0.05$) negative relationships. For the dispensable amino acids, with the exception of cystine and glycine, there were significant ($P < 0.05$) negative relationships. Not including RB 4, the linear relationships between the apparent ileal digestibilities of CP and amino acids and NDF-associated CP contents are presented in Table 3-6. Again, with the exception of methionine, cystine, and glycine, the negative linear relationships were significant ($P < 0.05$). Not including RB 4, more variation in amino acid digestibility was accounted for by the content of NDF-associated CP in the sources of RB. The regression equations show that as the level of CP associated with NDF in RB increases, there is a significant decrease in the apparent ileal digestibility of CP and most amino acids. This suggests that CP and amino acids associated with NDF from RB are not easily digested. Furthermore, it is evident that a relatively large proportion of CP is sequestered in the NDF fraction of some sources of RB. In studies with diets containing varying levels of wheat bran, shorts, and flour, Huang et al. (2001) reported negative correlations between the ileal digestibilities of CP and most of the amino acids and the content of CP associated with NDF.

It can be concluded that CP and amino acids associated with NDF in RB make a substantial contribution to the total CP and amino acid content in RB. Also, the CP content associated with the NDF fraction in RB is a useful indicator of AA and CP

digestibility as there are significant negative correlations between these two measurements.

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Table 3-1. Contents[a] (%) of crude protein and amino acids associated with neutral detergent fiber and their contribution (%) to the total crude protein and amino acid content in soybean meal, corn, and the sources of rice bran

	Ingredient													
	Soybean meal		Corn		Rice bran 1		Rice bran 2		Rice bran 3		Rice bran 4		Rice bran 5	
	Cont[b]	Contr	Cont	Contr	Cont	Contr	Cont	Contr	Cont	Contr	Cont	Contr	Cont	Contr
Crude protein	7.49	1.59	7.51	7.27	9.43	16.00	16.58	20.47	7.87	17.05	12.39	1.94	12.43	17.54
Amino acids														
Indispensable														
Arginine	0.35	1.03	0.34	7.13	0.55	11.02	1.01	15.16	0.41	10.90	0.43	0.93	0.72	12.68
Histidine	0.22	1.71	0.21	7.66	0.20	12.02	0.36	15.33	0.15	11.69	0.17	1.07	0.27	13.79
Isoleucine	0.25	1.28	0.20	5.97	0.33	17.11	0.60	22.91	0.27	16.53	0.32	1.55	0.43	18.61
Leucine	0.45	1.28	0.47	4.06	0.67	16.87	1.16	21.83	0.56	16.84	0.61	1.38	0.89	18.62
Lysine	0.41	1.47	0.27	8.87	0.32	12.26	0.60	17.71	0.26	11.88	0.18	0.85	0.42	14.11
Methionine	0.18	2.88	0.27	12.09	0.28	24.68	0.38	24.67	0.20	20.78	0.28	1.92	0.28	21.02
Phenylalanine	0.28	1.21	0.24	4.93	0.43	17.49	0.75	22.65	0.37	28.83	0.39	1.38	0.57	19.15
Threonine	0.27	1.43	0.32	8.76	0.37	17.47	0.61	21.12	0.32	17.69	0.28	1.30	0.48	18.85
Valine	0.33	1.63	0.32	7.00	0.49	16.19	0.87	21.72	0.41	16.15	0.45	1.50	0.64	18.36
Dispensable														
Alanine	0.29	1.45	0.33	4.64	0.53	15.40	0.91	19.37	0.45	15.74	0.44	1.30	0.69	17.10
Aspartic acid	0.56	1.05	0.41	5.85	0.81	17.13	1.35	20.03	0.68	17.53	0.59	1.16	1.03	18.01
Cystine	0.09	1.35	0.14	6.90	0.17	16.65	0.34	22.07	0.17	19.43	0.21	1.60	0.25	19.95
Glutamic acid	0.84	1.00	0.87	4.71	1.03	13.82	1.89	17.47	0.81	13.81	1.28	1.31	1.42	15.04
Glycine	0.42	2.17	0.33	8.55	0.53	17.09	0.85	20.25	0.45	17.53	0.34	1.25	0.68	18.47
Serine	0.36	1.46	0.27	5.43	0.41	15.27	0.72	20.11	0.35	16.21	0.35	1.20	0.56	17.44
Proline	0.37	1.53	0.50	5.53	0.46	17.63	0.77	22.47	0.41	18.99	0.40	1.42	0.63	19.61

[a]Contents (%, DM basis) of NDF were 11.21, 9.85, 26.44, 25.97, 34.28, 2.13, and 25.53 for soybean meal, corn, rice bran 1, rice bran 2, rice bran 3, rice bran 4, and rice bran 5, respectively.

[b]Contents in percentage

[c]Contributions in percentage

Table 3-2. Contents[a] (%) of crude protein and amino acids associated with neutral detergent fiber and their contribution (%) to the total crude protein and amino acid content in the experimental diets

	Diet											
	A		B		C		D		E		F	
Soybean meal	19.90		19.90		19.90		19.90		19.90		26.30	
Corn, %	53.80		53.80		53.80		53.80		53.80		71.03	
Rice bran, %	23.10		23.10		23.10		23.10		23.10		-	
Item	Cont[b]	Contr[c]	Cont	Contr	Cont	Contr	Cont	Contr	Cont	Contr	Cont	Contr
Crude protein	8.06	5.81	11.31	7.32	7.31	5.80	7.23	2.64	9.37	5.36	7.56	3.03
Amino acids												
Indispensable												
Arginine	0.40	4.12	0.59	5.39	0.33	3.81	0.30	1.65	0.42	3.73	0.31	2.15
Histidine	0.20	5.09	0.27	6.19	0.17	4.83	0.21	2.89	0.22	4.86	0.22	3.54
Isoleucine	0.27	4.87	0.37	5.89	0.24	4.55	0.23	2.10	0.27	4.06	0.22	2.31
Leucine	0.56	4.58	0.77	5.81	0.50	4.43	0.50	2.08	0.60	4.19	0.49	2.27
Lysine	0.26	3.75	0.38	4.96	0.23	3.62	0.23	1.80	0.27	3.40	0.25	2.28
Methionine	0.15	6.09	0.22	7.98	0.14	6.09	0.15	3.10	0.16	5.52	0.12	3.08
Phenylalanine	0.33	4.76	0.46	6.07	0.30	4.72	0.27	1.98	0.35	4.36	0.25	2.16
Threonine	0.32	5.85	0.43	7.20	0.29	5.79	0.29	2.79	0.34	5.46	0.29	3.30
Valine	0.41	6.05	0.56	7.31	0.37	5.82	0.35	2.73	0.42	5.18	0.34	3.10
Dispensable												
Alanine	0.41	5.27	0.58	6.82	0.37	5.21	0.34	2.32	0.44	4.86	0.33	2.63
Aspartic acid	0.60	4.45	0.83	5.45	0.54	4.34	0.44	1.65	0.63	4.01	0.44	1.97
Cystine	0.15	6.29	0.23	8.58	0.15	6.93	0.15	3.20	0.17	6.04	0.14	3.70
Glutamic acid	0.88	3.57	1.25	4.62	0.76	3.38	0.87	1.76	0.98	3.44	0.82	1.93
Glycine	0.43	6.98	0.57	8.27	0.39	6.94	0.35	3.08	0.46	6.42	0.36	3.88
Serine	0.34	4.80	0.47	6.14	0.30	4.72	0.29	2.10	0.37	4.61	0.29	2.56
Proline	0.48	5.52	0.59	6.59	0.45	5.59	0.48	2.88	0.51	4.86	0.50	3.28

[a]Contents (%, DM basis) of NDF were 12.99, 13.06, 14.78, 7.03, 11.72, and 8.20 for diets A, B, C, D, and E, respectively.
[b]Contents in percentage
[c]Contributions in percentage

Table 3-3. Contribution (%) of crude protein and amino acids associated with neutral detergent fiber to the total crude protein and amino acid contents in ileal digesta[a]

	Diet					
	A	B	C	D	E	F
Soybean meal, %:	19.90	19.90	19.90	19.90	19.90	26.30
Corn, %:	53.80	53.80	53.80	53.80	53.80	71.03
Rice bran, %:	23.10	23.10	23.10	23.10	23.10	-
Item						
Crude protein	12.69	12.77	12.12	8.88	11.38	8.53
Amino acids						
Indispensable						
Arginine	12.09	12.23	11.48	8.22	10.33	7.47
Histidine	12.09	12.78	11.54	9.10	11.73	10.23
Isoleucine	11.32	11.69	11.16	7.54	9.58	7.00
Leucine	11.80	12.20	11.87	7.02	9.81	6.58
Lysine	12.45	11.79	12.51	10.09	11.58	10.26
Methionine	14.88	18.34	13.53	10.80	13.86	10.23
Phenylalanine	11.66	12.10	12.02	6.62	10.16	6.25
Threonine	11.18	11.91	11.57	7.89	10.18	8.11
Valine	12.89	12.89	12.46	8.28	11.12	7.76
Dispensable						
Alanine	13.73	13.40	13.60	9.13	11.88	8.68
Aspartic acid	10.31	10.25	10.49	6.46	9.09	5.82
Cystine	11.28	11.79	11.48	6.65	9.64	7.16
Glutamic acid	9.62	10.10	9.47	7.07	8.52	6.52
Glycine	13.43	12.38	12.55	8.11	11.48	8.59
Serine	10.74	11.35	11.15	6.79	9.29	7.16
Proline	12.56	13.95	13.66	8.66	12.18	8.75

[a]Neutral detergent fiber contents (%, DM basis) were 12.99, 13.06, 14.78, 7.03, 11.72, and 8.20 for diets A, B, C, D, and E, respectively.

Table 3-4. The ileal digestibilities (%) of neutral detergent fiber and crude protein and amino acids associated with neutral detergent fiber in the experimental diets

	A	B	C	D	E	F	
Soybean meal, %:	19.90	19.90	19.90	19.90	19.90	26.30	
Corn, %:	53.80	53.80	53.80	53.80	53.80	71.03	
Rice bran, %:	23.10	23.10	23.10	23.10	23.10	-	
Item							SEM[a]
NDF	16.0[b]	-3.6[c]	4.2[c]	-8.1[c]	0.9[c]	-25.4[d]	3.75
Crude protein	50.65[b]	46.02[b]	39.15[c]	17.04[e]	47.19[b]	28.59[b]	1.95
Amino acids							
Indispensable							
Arginine	69.20[b]	64.59[bc]	58.87[c]	39.94[e]	63.82[bc]	51.46[d]	2.08
Histidine	62.20[b]	55.23[c]	53.04[c]	42.52[d]	56.46[c]	43.95[d]	1.32
Isoleucine	59.04[b]	51.93[c]	48.77[c]	29.50[e]	51.06[c]	39.20[d]	1.75
Leucine	61.75[b]	54.03[c]	50.18[d]	43.79[e]	57.06[c]	49.24[d]	1.09
Lysine	44.01[b]	45.02[b]	31.27[c]	-12.02[e]	31.49[c]	7.20[d]	2.90
Methionine	65.47[b]	54.48[c]	57.80[c]	41.15[d]	59.74[c]	46.01[d]	1.88
Phenylalanine	57.16[b]	50.83[c]	45.71[d]	35.27[e]	52.34[c]	38.97[e]	1.34
Threonine	54.51[b]	47.80[c]	44.39[c]	26.69[d]	48.02[c]	28.65[d]	1.34
Valine	59.71[b]	53.75[c]	49.94[c]	36.68[e]	53.20[c]	40.99[d]	1.29
Dispensable							
Alanine	53.57[b]	49.39[b]	41.74[c]	17.87[e]	47.07[bc]	25.90[d]	2.12
Aspartic acid	55.21[b]	48.82[bc]	42.93[c]	20.15[e]	48.09[bc]	32.08[d]	2.05
Cystine	60.81[b]	53.46[c]	54.23[c]	47.15[d]	58.22[b]	47.75[d]	1.23
Glutamic acid	64.69[b]	57.32[cd]	53.99[d]	40.57[bc]	60.39[bc]	45.71[e]	1.53
Glycine	52.15[b]	45.17[cd]	41.86[d]	23.82[e]	46.50[c]	25.69[e]	1.22
Serine	58.83[b]	51.87[c]	47.31[d]	31.66[f]	54.02[c]	35.86[e]	1.26
Proline	59.17[b]	47.32[d]	47.49[d]	41.07[e]	51.42[c]	43.99[de]	1.06

[a]Standard error of the mean.

[b,c,d,e,f]Means in the same row with different superscript letters differ ($P < 0.05$).

Table 3-5. The linear relationships between apparent ileal digestibilities of crude protein and amino acids and neutral detergent fiber associated crude protein[a] in the sources of rice bran

Item	Regression equations	r^2	P[b]	P[c]
Crude protein	Y[d] = 70.38 – 1.22X[e]	0.0596	< 0.0001	0.1936
Amino acids				
Indispensable				
Arginine	Y = 88.54 – 0.61X	0.1384	< 0.0001	0.0430
Histidine	Y = 91.29 – 1.30X	0.2495	< 0.0001	0.0050
Isoleucine	Y = 87.25 – 1.64X	0.1544	< 0.0001	0.0317
Leucine	Y = 84.45 – 1.62X	0.1873	< 0.0001	0.0169
Lysine	Y = 91.21 – 1.62X	0.1735	< 0.0001	0.0220
Methionine	Y = 80.37 – 0.87X	0.1051	< 0.0001	0.0805
Phenylalanine	Y = 84.63 – 1.47X	0.1666	< 0.0001	0.0252
Threonine	Y = 98.08 – 1.93X	0.1605	< 0.0001	0.0282
Valine	Y = 87.19 – 1.30X	0.1341	< 0.0001	0.0466
Tryptophan	Y = 91.84 – 1.79X	0.1516	< 0.0001	0.0334
Dispensable				
Alanine	Y = 87.89 – 1.32X	0.1468	< 0.0001	0.0366
Aspartic acid	Y = 87.31 – 1.65X	0.1457	< 0.0001	0.0374
Cystine	Y = 88.37 – 1.56X	0.0854	< 0.0001	0.1171
Glutamic acid	Y = 88.09 – 1.50X	0.1353	< 0.0001	0.0455
Glycine	Y = 87.72 – 1.60X	0.0909	< 0.0001	0.1053
Serine	Y = 88.78– 1.53X	0.1655	< 0.0001	0.0257
Proline	Y = 85.29 – 1.73X	0.1023	< 0.0001	0.0848

[a] Content of CP associated with NDF in the RB varieties ranged from 7.87 to 16.58%.
[b] The probabilities of significance for the intercepts of the regression equations.
[c] The probabilities of significance for the slopes of the regression equations.
[d] Y represents digestibility coefficient (%).
[e] X represents content (%) of CP associated with NDF.

Table 3-6. The linear relationships between apparent ileal digestibilities of crude protein and amino acids and neutral detergent fiber associated crude protein[a] in the sources of rice bran (excluding rice bran 4)

Item	Regression equations	r^2	P[b]	P[c]
Crude protein	Y[d] = 70.01 – 1.40X[e]	0.0919	< 0.0001	0.1498
Amino acids				
Indispensable				
Arginine	Y = 88.46 – 0.65X	0.1734	< 0.0001	0.0429
Histidine	Y = 91.12 – 1.39X	0.3314	< 0.0001	0.0032
Isoleucine	Y = 87.02 – 1.75X	0.2042	< 0.0001	0.0266
Leucine	Y = 84.12 – 1.79X	0.2731	< 0.0001	0.0088
Lysine	Y = 91.24 – 1.61X	0.2177	< 0.0001	0.0215
Methionine	Y = 80.27 – 0.92X	0.1354	< 0.0001	0.0769
Phenylalanine	Y = 84.25 – 1.66X	0.2728	< 0.0001	0.0088
Threonine	Y = 97.65 – 2.15X	0.2544	< 0.0001	0.0120
Valine	Y = 86.98 – 1.41X	0.1862	< 0.0001	0.0353
Tryptophan	Y = 91.83 – 1.79X	0.1805	< 0.0001	0.0385
Dispensable				
Alanine	Y = 87.69 – 1.42X	0.2009	< 0.0001	0.0280
Aspartic acid	Y = 86.85 – 1.88X	0.2606	< 0.0001	0.0108
Cystine	Y = 87.93 – 1.78X	0.1324	< 0.0001	0.0805
Glutamic acid	Y = 87.78 – 1.66X	0.1898	< 0.0001	0.0333
Glycine	Y = 87.34 – 1.79X	0.1455	< 0.0001	0.0659
Serine	Y = 88.41– 1.71X	0.2824	< 0.0001	0.0075
Proline	Y = 84.96 – 1.90X	0.1392	< 0.0001	0.0725

[a] Content of CP associated with NDF in the RB varieties ranged from 7.87 to 16.58%.
[b] The probabilities of significance for the intercepts of the regression equations.
[c] The probabilities of significance for the slopes of the regression equations.
[d] Y represents digestibility coefficient (%).
[e] X represents content (%) of CP associated with NDF.

Table 3-7. The contribution (%) of crude protein and selected amino acids associated with neutral detergent fiber from rice bran to the total content of undigested crude protein and amino acids from rice bran in ileal digesta

Item	Rice bran				
	1	2	3	4	5
Crude protein	12.42	7.41	9.55	8.26	7.36
Amino acids					
Lysine	17.50	6.97	11.50	10.77	9.80
Methionine	12.92	12.34	8.26	6.98	11.36
Threonine	26.04	11.22	17.90	17.19	14.65

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

A. Discussion

Rice bran (**RB**) is a by-product of the rice milling industry. As rice (*Oryza sativa*) is a staple food for over half the global population, RB is produced in considerable quantities, and while its benefits are being discovered in human nutrition (Slavin and Lampe, 1992), it is most commonly utilized as a feedstuff in animal agriculture.

Rice bran contains approximately 12 to 16 % CP, 15 to 20 % crude fat (Juliano, 1985), and 23 % NDF (NRC, 1998). The contents of the critical amino acids in diet formulation (i.e. amino acids that are often limiting), namely lysine, methionine, threonine and tryptophan, are approximately 0.57, 0.26, 0.48, and 0.14 %, respectively (NRC, 1998). Many studies have been carried out previously to determine the nutritive value of RB for poultry (e.g. Kratzer et al, 1974; Majun and Payne, 1977; Adrizal and Sell, 1996) and for pigs (e.g. Baird et al, 1975; Brooks and Lumanta, 1975b). However, there is a scarcity of information on the digestibility of GE and CP and especially on the ileal amino acid digestibility values in RB and the variability in these values among different sources of RB for pigs.

Therefore, studies were performed to determine the digestibility values of GE and CP and the apparent ileal digestibility of amino acids in different sources of RB. The fecal analysis method was used to determine the digestibility of GE. The ileal rather than fecal analysis method was used to determine the amino acid digestibilities for reasons of

fermentation of protein and other nitrogen-containing compounds by the microflora in the large intestine (Sauer and Ozimek, 1986). Furthermore, the difference rather than direct method was chosen to determine the apparent ileal amino acid digestibility values in RB. The direct method will underestimate digestibility values in ingredients such as RB that have a relatively low content of CP and amino acids (Fan and Sauer, 1995).

The digestibility of GE was highest for RB 4 (86.9 %) which had the lowest NDF content (2.1 %) among the RB samples. This sample can be considered an atypical source of RB. The very low content of NDF in the sample can be attributed to the fact that this particular sample consisted mainly of "polish", which is largely made up of starchy endosperm removed from the rice kernel during processing. The digestibilities of GE of the other samples ranged from 60.5 to 65.8 % with NDF contents ranging from 25.5 to 34.3 %. There was considerable variation in the apparent ileal amino acid digestibilities among the RB samples. Not including RB 4, for the critical amino acids, the apparent ileal digestibilities of lysine, ranged from 62.6 to 82.2 %, of methionine from 61.4 to 76.0 %, of threonine from 58.4 to 82.8 %, and of tryptophan from 58.8 to 82.6 %.

Previous studies carried out with wheat shorts (Huang et al., 1999), canola meal (Fan et al., 1996), and wheat (Fan et al., 2001) have shown significant negative correlations between the apparent ileal CP and amino acid digestibilities and the NDF content of these ingredients. The lower CP and amino acid digestibilities with increasing fiber content may be attributed to an increase in the rate of passage of digesta (Stanogias and Pearce, 1985), adsorption of amino acids and peptides to fiber, increased losses of endogenous CP and amino acids, and a lower digestibility of CP and amino acids

associated with fiber (Donangelo and Eggum, 1985). Not including RB 4, the atypical source of RB, there were no significant correlations between the NDF content (25.5 to 34.3 %) and the apparent ileal amino acid digestibilities of lysine, the sulphur-containing amino acids, threonine, and tryptophan.

Linear relationships were also established between the dietary crude fat (**CF**), as well as between the dietary ash and CP contents and amino acid digestibility values. Not including RB 4, with the exception of tryptophan, there were significant positive correlations between the amino acid digestibility values and the CF content. Not including RB 4, there were positive relationships between the ash content (11.0 to 21.0 %) and the amino acid digestibility values. Not including RB 4, there were negative correlations between the dietary CP content (15.6 to 21.0 %) and the amino acid digestibility values.

The results of these studies indicate that some of the variation in amino acid digestibility values between samples of RB can be attributed to differences in their contents of CF, ash, and CP but not to differences in NDF content.

It should be noted that the sources of RB that were used in this study originated from rice cultivated at different locations under different environmental conditions. Further, the samples of RB were obtained under different milling conditions and further defatted or extruded. In order to refine these studies, it is necessary to obtain rice from different locations, subject a portion of each to different milling conditions to produce RB, and from this take a portion to subject to different treatments (i.e. extrusion cooking or defatting) and then to compare the results. The aforementioned approach, although ideal, is highly impractical.

The ileal digestibilities of amino acids associated with the NDF fraction are likely to be relatively low because the digestive enzymes have limited access to the cell wall components and cell walls (Bjergegaard et al., 1991). Huang et al. (2001) reported that a considerable proportion of amino acids in wheat shorts are associated with the NDF fraction and of which the digestibilities were relatively low. Therefore, studies were also carried out to determine the proportion of amino acids associated with the NDF fraction and the digestibilities of the NDF-associated amino acids in the sources of RB.

Not including RB 4, the contribution of CP associated with NDF to total CP in the sources of RB ranged from 16.0 to 20.5 %. The contributions of lysine, methionine, and threonine associated with NDF to their total contents in RB ranged from 11.9 to 17.7 %, from 20.8 to 24.7 %, and from 17.5 to 21.2 %, respectively. Of the RB-containing diets, not including diet D which contained RB 4, the ileal digestibility of NDF-associated CP ranged from 39.2 to 50.7 %. The digestibilities of NDF-associated lysine, methionine, and threonine ranged from 31.3 to 45.0 %, from 54.5 to 65.5 %, and from 44.4 to 54.1 %. Simple linear regression equations were established between the apparent ileal CP and amino acid digestibilities and the content of CP associated with NDF in the sources of RB. Not including RB 4, there were significant negative correlations for most amino acids.

B. Conclusions

In conclusion:

1. The apparent ileal digestibilities of amino acids in the different sources of RB were relatively low and quite variable.
2. The crude protein, crude fat, and ash contents in the sources of RB were partially responsible for the variation in amino acid digestibilities, while NDF content was not.
3. Considerable proportions of CP and amino acids in the different sources of RB were associated with NDF. The ileal digestibilities of amino acids associated with NDF were relatively low.
4. The content of CP associated with NDF is a relatively good indicator of apparent ileal amino acid digestibility, as these two parameters were significantly correlated for most amino acids.

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APPENDIX

Table 1. The linear relationships[a] between apparent ileal digestibilities of crude protein and amino acids and neutral detergent fiber content in different sources of rice bran

Item	Regression equations	r^2	P[b]	P[c]
Crude protein	Y[d] = 67.2 - 0.49X[e]	0.1255	< 0.0001	0.0548
Amino acids				
Indispensable				
Arginine	Y = 83.44 – 0.09X	0.0403	< 0.0001	0.2872
Histidine	Y = 79.51 – 0.15X	0.0458	< 0.0001	0.2561
Isoleucine	Y = 73.07 – 0.22X	0.0368	< 0.0001	0.3100
Leucine	Y = 73.35 – 0.35X	0.1127	< 0.0001	0.0697
Lysine	Y = 70.35 + 0.08X	0.0059	< 0.0001	0.6861
Methionine	Y = 73.07 - 0.13X	0.0304	< 0.0001	0.3565
Phenylalanine	Y = 76.80 – 0.41X	0.1724	< 0.0001	0.0225
Threonine	Y = 85.64 – 0.45X	0.1137	< 0.0001	0.0684
Valine	Y = 76.72 – 0.21X	0.0460	< 0.0001	0.2552
Tryptophan	Y = 70.24 + 0.03X	0.0004	< 0.0001	0.9114
Dispensable				
Alanine	Y = 77.35 – 0.22X	0.0527	< 0.0001	0.2224
Aspartic acid	Y = 79.37 – 0.50X	0.1763	< 0.0001	0.0209
Cystine	Y = 82.53 – 0.54X	0.1378	< 0.0001	0.0434
Glutamic acid	Y = 78.72 – 0.36X	0.1030	< 0.0001	0.0838
Glycine	Y = 79.47 – 0.46X	0.0992	< 0.0001	0.0900
Serine	Y = 80.15 – 0.41X	0.1546	< 0.0001	0.0316
Proline	Y = 73.93 – 0.39X	0.0694	< 0.0001	0.1595

[a] Content of NDF in the RB varieties ranged from 2.1 to 34.3%.
[b] The probabilities of significance for the intercepts of the regression equations.
[c] The probabilities of significance for the slopes of the regression equations.
[d] Y represents digestibility coefficient (%).
[e] X represents content (%) of NDF in the rice bran.

Table 2. The linear relationships[a] between apparent ileal digestibilities of crude protein and amino acids and neutral detergent fiber content in different sources of rice bran (excluding rice bran 4)

Item	Regression equations	r^2	P[b]	P[c]
Crude protein	Y[d] = 79.73 - 0.93X[e]	0.0472	0.0043	0.3079
Amino acids				
Indispensable				
Arginine	Y = 85.96 – 0.18X	0.0156	0.0001	0.5615
Histidine	Y = 70.28 + 0.17X	0.0058	0.0001	0.7225
Isoleucine	Y = 61.73 + 0.18X	0.0025	0.0089	0.8179
Leucine	Y = 60.98 + 0.09X	0.0008	0.0040	0.8972
Lysine	Y = 70.19 + 0.09X	0.0008	0.0013	0.8978
Methionine	Y = 81.21 - 0.41X	0.0322	0.0001	0.4012
Phenylalanine	Y = 63.89 + 0.04X	0.0002	0.0015	0.9477
Threonine	Y = 65.66 + 0.25X	0.0041	0.0109	0.7654
Valine	Y = 68.54 + 0.08X	0.0007	0.0010	0.9048
Tryptophan	Y = 74.90 - 0.14X	0.0012	0.0041	0.8707
Dispensable				
Alanine	Y = 75.43 – 0.15X	0.0027	0.0003	0.8108
Aspartic acid	Y = 63.65 + 0.05X	0.0002	0.0050	0.9433
Cystine	Y = 88.35 – 0.75X	0.0277	0.0032	0.4368
Glutamic acid	Y = 77.76 – 0.33X	0.0087	0.0012	0.6645
Glycine	Y = 80.74 – 0.50X	0.0136	0.0050	0.5873
Serine	Y = 68.74 – 0.01X	0.0000	0.0009	0.9920
Proline	Y = 77.94 – 0.53X	0.0130	0.0109	0.5964

[a] Content of NDF in the RB varieties ranged from 25.5 to 34.3%.
[b] The probabilities of significance for the intercepts of the regression equations.
[c] The probabilities of significance for the slopes of the regression equations.
[d] Y represents digestibility coefficient (%).
[e] X represents content (%) of NDF in the rice bran.

Table 3. The linear relationships[a] between apparent ileal digestibilities of crude protein and amino acids and crude fat content in different sources of rice bran

Item	Regression equations	r^2	P[b]	P[c]
Crude protein	Y[d] = 50.6 + 0.35X[e]	0.0445	< 0.0001	0.2633
Amino acids				
Indispensable				
Arginine	Y = 78.53 + 0.18X	0.1089	< 0.0001	0.0749
Histidine	Y = 71.20 + 0.31X	0.1251	< 0.0001	0.0552
Isoleucine	Y = 61.56 + 0.42X	0.0893	< 0.0001	0.1086
Leucine	Y = 60.38 + 0.33X	0.0676	< 0.0001	0.1654
Lysine	Y = 63.83 + 0.54X	0.1725	< 0.0001	0.0225
Methionine	Y = 65.59 + 0.29X	0.1036	< 0.0001	0.0829
Phenylalanine	Y = 63.23 + 0.27X	0.0497	< 0.0001	0.2363
Threonine	Y = 70.20 + 0.33X	0.0431	< 0.0001	0.2712
Valine	Y = 66.94 + 0.32X	0.0731	< 0.0001	0.1485
Tryptophan	Y = 61.44 + 0.60X	0.1548	< 0.0001	0.0315
Dispensable				
Alanine	Y = 67.38 + 0.32X	0.0768	< 0.0001	0.1383
Aspartic acid	Y = 63.95 + 0.26X	0.0320	< 0.0001	0.3439
Cystine	Y = 64.75 + 0.34X	0.0371	< 0.0001	0.3076
Glutamic acid	Y = 65.09 + 0.35X	0.0645	< 0.0001	0.1757
Glycine	Y = 63.64 + 0.34X	0.0373	< 0.0001	0.3063
Serine	Y = 66.71 + 0.27X	0.0452	< 0.0001	0.2593
Proline	Y = 56.93 + 0.52X	0.0812	< 0.0001	0.1269

[a] Content of crude fat in the sources of rice bran ranged from 4.1 to 24.4%.
[b] The probabilities of significance for the intercepts of the regression equations.
[c] The probabilities of significance for the slopes of the regression equations.
[d] Y represents digestibility coefficient (%).
[e] X represents content (%) of crude fat in the rice bran.

Table 4. The linear relationships[a] between apparent ileal digestibilities of crude protein and amino acids and crude fat content in different sources of rice bran (excluding rice bran 4)

Item	Regression equations	r^2	P[b]	P[c]
Crude protein	Y[d] = 36.51 + 0.95X[e]	0.2598	< 0.0001	0.0109
Amino acids				
Indispensable				
Arginine	Y = 74.62 + 0.35X	0.3047	< 0.0001	0.0052
Histidine	Y = 63.52 + 0.64X	0.4257	< 0.0001	0.0005
Isoleucine	Y = 51.01 + 0.87X	0.3052	< 0.0001	0.0051
Leucine	Y = 47.89 + 0.86X	0.3861	< 0.0001	0.0012
Lysine	Y = 59.66 + 0.72X	0.2661	< 0.0001	0.0099
Methionine	Y = 60.02 + 0.53X	0.2710	< 0.0001	0.0091
Phenylalanine	Y = 49.85 + 0.84X	0.4278	< 0.0001	0.0005
Threonine	Y = 54.69 + 0.99X	0.3342	< 0.0001	0.0031
Valine	Y = 57.93 + 0.70X	0.2870	< 0.0001	0.0070
Tryptophan	Y = 55.65 + 0.85X	0.2490	< 0.0001	0.0131
Dispensable				
Alanine	Y = 58.67 + 0.69X	0.2901	< 0.0001	0.0066
Aspartic acid	Y = 48.44 + 0.92X	0.3828	< 0.0001	0.0013
Cystine	Y = 48.93 + 1.02X	0.2667	< 0.0001	0.0098
Glutamic acid	Y = 52.92 + 0.87X	0.3174	< 0.0001	0.0041
Glycine	Y = 49.43 + 0.95X	0.2505	< 0.0001	0.0128
Serine	Y = 53.56 + 0.83X	0.4030	< 0.0001	0.0009
Proline	Y = 42.51 + 1.13X	0.3033	< 0.0001	0.0053

[a] Content of crude fat in the sources of rice bran ranged from 4.1 to 24.4%.
[b] The probabilities of significance for the intercepts of the regression equations.
[c] The probabilities of significance for the slopes of the regression equations.
[d] Y represents digestibility coefficient (%).
[e] X represents content (%) of crude fat in the rice bran.

Table 5. The linear relationships[a] between apparent ileal digestibilities of crude protein and amino acids and ash content in different sources of rice bran

Item	Regression equations	r^2	P[b]	P[c]
Crude protein	Y[d] = 53.88 + 0.17X[e]	0.0037	< 0.0001	0.7504
Amino acids				
Indispensable				
Arginine	Y = 78.92 + 0.19X	0.0419	< 0.0001	0.2777
Histidine	Y = 74.22 + 0.14X	0.0089	< 0.0001	0.6199
Isoleucine	Y = 66.66 + 0.11X	0.0021	< 0.0001	0.8101
Leucine	Y = 65.46 - 0.0008X	0.0000	< 0.0001	0.9984
Lysine	Y = 63.35 + 0.68X	0.0995	< 0.0001	0.0895
Methionine	Y = 65.43 + 0.36X	0.0573	< 0.0001	0.2025
Phenylalanine	Y = 69.40 - 0.15X	0.0059	< 0.0001	0.6875
Threonine	Y = 77.13 - 0.13X	0.0024	< 0.0001	0.7960
Valine	Y = 71.01 + 0.07X	0.0013	< 0.0001	0.8512
Tryptophan	Y = 60.55 + 0.78X	0.0958	< 0.0001	0.0961
Dispensable				
Alanine	Y = 69.03 + 0.25X	0.0177	< 0.0001	0.4839
Aspartic acid	Y = 70.66 - 0.20X	0.0074	< 0.0001	0.6513
Cystine	Y = 67.93 + 0.17X	0.0032	< 0.0001	0.7680
Glutamic acid	Y = 68.06 + 0.18X	0.0067	< 0.0001	0.6667
Glycine	Y = 66.51 + 0.19X	0.0041	< 0.0001	0.7358
Serine	Y = 71.60 - 0.06X	0.0007	< 0.0001	0.8865
Proline	Y = 61.72 + 0.25X	0.0068	< 0.0001	0.6646

[a] Content of ash in the sources of rice bran ranged from 4.8 to 21.0%.
[b] The probabilities of significance for the intercepts of the regression equations.
[c] The probabilities of significance for the slopes of the regression equations.
[d] Y represents digestibility coefficient (%).
[e] X represents content (%) of ash in the rice bran.

Table 6. The linear relationships[a] between apparent ileal digestibilities of crude protein and amino acids and ash content in different sources of rice bran (excluding rice bran 4)

Item	Regression equations	r^2	P[b]	P[c]
Crude protein	Y[d] = 22.47 + 0.06X[e]	0.2631	0.0640	0.0104
Amino acids				
Indispensable				
Arginine	Y = 69.25 + 0.77X	0.3216	< 0.0001	0.0039
Histidine	Y = 59.49 + 1.02X	0.2388	< 0.0001	0.0154
Isoleucine	Y = 48.20 + 1.22X	0.1307	0.0001	0.0826
Leucine	Y = 41.67 + 1.43X	0.2330	< 0.0001	0.0169
Lysine	Y = 51.59 + 1.38X	0.2154	< 0.0001	0.0223
Methionine	Y = 50.34 + 1.26X	0.3379	< 0.0001	0.0029
Phenylalanine	Y = 45.25 + 1.30X	0.2234	< 0.0001	0.0197
Threonine	Y = 48.75 + 1.58X	0.1818	0.0003	0.0377
Valine	Y = 54.71 + 1.05X	0.1385	< 0.0001	0.0733
Tryptophan	Y = 43.70 + 1.80X	0.2416	0.0005	0.0147
Dispensable				
Alanine	Y = 48.96 + 1.46X	0.2814	< 0.0001	0.0077
Aspartic acid	Y = 41.84 + 1.53X	0.2298	0.0002	0.0178
Cystine	Y = 31.63 + 2.35X	0.3085	0.0134	0.0048
Glutamic acid	Y = 41.19 + 1.80X	0.2990	0.0002	0.0057
Glycine	Y = 33.92 + 2.15X	0.2791	0.0076	0.0080
Serine	Y = 45.69 + 1.50X	0.2886	< 0.0001	0.0068
Proline	Y = 32.35 + 2.01X	0.2090	0.0219	0.0247

[a] Content of ash in the sources of rice bran ranged from 11.0 to 21.0%.
[b] The probabilities of significance for the intercepts of the regression equations.
[c] The probabilities of significance for the slopes of the regression equations.
[d] Y represents digestibility coefficient (%).
[e] X represents content (%) of ash in the rice bran.

Table 7. The linear relationships[a] between apparent ileal digestibilities of crude protein and amino acids and crude protein content in different sources of rice bran

Item	Regression equations	r^2	P[b]	P[c]
Crude protein	Y[d] = 102.61 – 2.77X[e]	0.2218	< 0.0001	0.0086
Amino acids				
Indispensable				
Arginine	Y = 97.02 – 0.93X	0.2315	< 0.0001	0.0071
Histidine	Y = 107.67 – 1.88X	0.3766	< 0.0001	0.0003
Isoleucine	Y = 108.72 – 2.42X	0.2436	< 0.0001	0.0056
Leucine	Y = 113.07 – 2.83X	0.4136	< 0.0001	0.0001
Lysine	Y = 96.80 – 1.46X	0.1022	< 0.0001	0.0850
Methionine	Y = 92.29 – 1.32X	0.1728	< 0.0001	0.0223
Phenylalanine	Y = 115.57 – 2.86X	0.4580	< 0.0001	0.0001
Threonine	Y = 134.38 – 3.50X	0.3819	< 0.0001	0.0003
Valine	Y = 106.11 – 2.03X	0.2369	< 0.0001	0.0064
Tryptophan	Y = 101.29 – 1.81X	0.1121	< 0.0001	0.0705
Dispensable				
Alanine	Y = 107.88 – 2.11X	0.2701	< 0.0001	0.0032
Aspartic acid	Y = 124.40 – 3.35X	0.4362	< 0.0001	0.0001
Cystine	Y = 126.72 - 3.36X	0.2885	< 0.0001	0.0022
Glutamic acid	Y = 116.50 – 2.73X	0.3252	< 0.0001	0.0010
Glycine	Y = 122.14 – 3.16X	0.2573	< 0.0001	0.0042
Serine	Y = 120.22 – 2.93X	0.4416	< 0.0001	0.0001
Proline	Y = 115.59 – 3.01X	0.2234	< 0.0001	0.0084

[a] Content of crude protein in the sources of rice bran ranged from 13.6 to 21.0%.
[b] The probabilities of significance for the intercepts of the regression equations.
[c] The probabilities of significance for the slopes of the regression equations.
[d] Y represents digestibility coefficient (%).
[e] X represents content (%) of CP in the rice bran.

Table 8. The linear relationships[a] between apparent ileal digestibilities of crude protein and amino acids and crude protein content in different sources of rice bran (excluding rice bran 4)

Item	Regression equations	r^2	P[b]	P[c]
Crude protein	Y[d] = 101.40 – 2.70X[e]	0.1493	0.0004	0.0622
Amino acids				
Indispensable				
Arginine	Y = 102.07 – 1.20X	0.2581	< 0.0001	0.0113
Histidine	Y = 116.38 – 2.34X	0.4138	< 0.0001	0.0007
Isoleucine	Y = 118.24 – 2.92X	0.2482	< 0.0001	0.0132
Leucine	Y = 117.27 – 3.05X	0.3494	< 0.0001	0.0024
Lysine	Y = 121.87 – 2.79X	0.2883	< 0.0001	0.0068
Methionine	Y = 100.64 – 1.76X	0.2159	< 0.0001	0.0221
Phenylalanine	Y = 114.71 – 2.82X	0.3446	< 0.0001	0.0026
Threonine	Y = 136.72 – 3.63X	0.3169	< 0.0001	0.0042
Valine	Y = 112.46 – 2.37X	0.2316	< 0.0001	0.0173
Tryptophan	Y = 127.58 – 3.20X	0.2524	< 0.0001	0.0124
Dispensable				
Alanine	Y = 116.25 – 2.56X	0.2831	< 0.0001	0.0074
Aspartic acid	Y = 121.82 – 3.22X	0.3347	< 0.0001	0.0031
Cystine	Y = 126.57 - 3.36X	0.2072	< 0.0001	0.0254
Glutamic acid	Y = 121.47 – 3.00X	0.2725	< 0.0001	0.0089
Glycine	Y = 125.05 – 3.31X	0.2185	< 0.0001	0.0213
Serine	Y = 121.08 – 2.98X	0.3733	< 0.0001	0.0015
Proline	Y = 122.76 – 3.40X	0.1947	0.0001	0.0309

[a] Content of crude protein in the sources of rice bran ranged from 15.6 to 21.0%.
[b] The probabilities of significance for the intercepts of the regression equations.
[c] The probabilities of significance for the slopes of the regression equations.
[d] Y represents digestibility coefficient (%).
[e] X represents content (%) of CP in the rice bran.